STUDIES OF THE MYCOFLORA OF SOME LEAD CONTAMINATED SOILS

by

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ABSTRACT

An ecological study of the mycoflora of some lead contaminated soils was undertaken. Five soils were sampled bimonthly over a two-year period. Two of the soils consisted of old mine 'Tailings', both of which had received an application of topsoil at some time. Two other soils were a result of the processing of the ore for marketing and the remaining soil sampled was part of an agricultural field nearby.

The numbers of fungi found in all the soils sampled were appreciably lower than those in normal soils. This was so even in the agricultural field which did not contain so much lead as the other soils, but contained many more isolates/gram than the other soils. A wide variety of species was isolated from the soils but only a few were major contributors to the mycoflora. All species isolated have been found previously in soils to varying extents. There were some differences between the soils investigated and normal soils, but these were not in the kind of species but their relative numbers. However, the differences were not sufficient to give rise to a distinctive flora associated with lead soils. Two fungi in particular occurred more than in normal soils - Coniothyrium fuckelii and Phoma herbarum. The latter seemed to be an important member of the mycoflora of the spoil heaps.

The soils were also studied for seasonal variation and the effect of depth. It was found that there was no apparent seasonal differences but numbers of both species and isolates were affected adversely by depth.

In addition to the ecological studies the effect of lead on the growth of Aspergillus fumigatus Fres. was investigated. On solid

media growth could occur at very high concentrations up to 1500 p.p.m. Most of the experiments were conducted using liquid media in which growth was prevented above 170 p.p.m. Germination of A. fumigatus spores can occur over a wide range of lead concentrations, but mycelial growth is much more limited. Tolerance of lead by the mycelium increases until after four days it has no effect on the growth of the mycelium. Lead would seem to be fungistatic rather than fungicidal, since it hinders growth but does not prevent it and once the lead is removed growth continues normally.

In addition to the effect of lead on the growth of A. fumigatus isolates of some species from spoil and non-lead-containing areas were compared for their ability to grow on lead-containing media. There was a varied reaction of the isolates which indicated that the fungal flora was composed of two types. One kind of fungus was normally lead tolerant while the other developed a higher lead tolerance in the spoil.

CONTENTS

INTRODUCTION

In recent years there has been concern over the increasing amount of lead in circulation in the environment. This is a relatively recent phenomenon as shown by the analysis of Greenland and Antarctic snow strata (Murozumi, Chow and Patterson 1969). They found that the lead content rose sharply after 1940 which coincides with the increase in burning lead alkyls in the motor car. Prior to 1940 most of the lead came from smelters, this accounted for the lead content after 1753 since when the amount gradually rose and this source was not reduced until this century. This was also confirmed by Ruhling and Tyler (1969) in a study using herbarium specimens of Hypnum cupressiforme and analysing them for their lead content.

Since a large proportion of the lead pollution has resulted from the motor car much research on the accumulation of lead in soils (Page and Ganje 1970) has been conducted in the vicinity of roads. Other researches have been concerned with the lead content in naturally occurring plants at the roadside, (Chow 1970), and also with various agricultural crops (Page, Ganje and Joshi 1971). The lead alkyl derivatives are combusted and some of the lead is given out in the fumes. The lead may fall at the roadside or may be carried long distances by wind and later fall with the precipitation. Ruhling and Tyler (1968) studied the amount of lead in plants and soils near and far from main roads. The results showed that near the roads the amount of lead in the soil is much greater than in the plant biomass. However from their results they concluded that "... only a minor part of the lead which is liberated by the cars on the combustion of lead petrol, will settle and accumulate in the vicinity of roads ..." indicating that the problem is not limited geographically. In the regional study

using mosses they found that the amount of lead in various areas was closely related to the mean annual precipitation and the nearness of any population centres. It would seem therefore that the lead from the old smelters had possibly carried over quite a wide area and been deposited in the soils and plants.

Lead may also occur in soils naturally. This is due to the movement of water which may have been in contact with lead ore underground. When man intervenes as in mining operations much more of the lead is released to the environment. This was due to the methods of extracting the finer ore fragments by washing. Further, the mines have to be drained and often the drainage water is contaminated and the fine particles may settle out at some point in the course of the stream. There is also a large amount of dust which contains lead, which may be produced by the mine or by the smelter, and this may spread over the adjacent area and also further afield where it may be deposited in precipitation.

Another feature of mining which produces a problem is the formation of large spoil heaps, which may extend over a wide area. These spoil heaps may consist of gangue, that is other minerals from the vein or some waste from the preparation of ore for marketing. These heaps contain varying amounts of lead depending on the efficacy of the extraction procedures, and many have been reworked this century. Lead has been mined in some way in several parts of Britain since the Roman Occupation and in some cases earlier (Kirkham 1968, Ford and Rieuwarts 1968). In Derbyshire and elsewhere many of the sites of earlier mining activity have long since ceased working and have become revegetated, generally with grasses, leaving mounds and sometimes old mine shafts as the only outward sign of any activity.

Old mine spoil heaps are generally considered to be poorly structured, to have a low water holding capacity and also to lack the major nutrients so making them inhospitable to plant colonisation (Ernst 1968). There have been several studies on the vegetation associated with heavy metal contaminated areas in general terms (Ernst 1968, Lower Swansea Valley Project 1967) and with reference to specific plants such as Festuca ovina (Wilkins 1957 and 1960) and Thlaspe alpestre L (Shimwell 1968). Agrostis species particularly A. tenuis are important colonisers of spoil from heavy metal workings (Jowett 1958 and 1964, Bradshaw 1952).

The populations of A. tenuis from mine spoil have been measured for metal tolerance in comparison with plants from normal habitats. It has been found that the plants from mine sites show tolerance to the metals present in the soil from which they came (Gregory and Bradshaw 1965). Variation in the degree of tolerance may be due in part to the adaptation of the plants to low calcium and phosphate levels found in lead mine spoil (Jowett 1964). There are differences in the form of the plants from different habitats. Wilkins (1957) showed that the toxicity of lead was reduced by the presence of calcium ions. He found that lead was toxic in very small quantities and neither plants from normal habitats nor those from lead mine spoil grew very much at a concentration of 100 p.p.m. of lead.

As most research concerning lead contamination has been concerned with grasses, because of the danger to livestock (Egan and O'Cuill 1969) and their ability to colonise toxic areas, and commercial crops, it was decided that it might prove interesting to investigate the fungal flora of lead contaminated soils. It was proposed to examine an area of recent industrial activity in order to see whether fungi were early colonisers and able to overcome the inhospitality of the lead spoil.

There have been many investigations of the mycoflora of soils from a wide variety of habitats many of these resulting in lists of fungi isolated (Bisby, James and Timonin 1933). The many investigations of soil populations have shown that there is a wide and varied fungal flora inhabiting soils. In addition there have been studies considering the position occupied by fungi in the soil (Jensen 1931) and their relation and adaptation to their habitat (Garrett 1951). Waksman (1944) comprehensively reviewed the knowledge of soil fungi and their growth in the soil as well as the position they hold in the soil environment. Chesters (1949) also reviewed some of the work which had been carried out and some of the problems facing the soil mycologist.

Later investigations studied a particular habitat in more detail than merely enumerating the fungi isolated with no reference to depth or other detail. Warcup (1951) conducted a study of five natural grassland soils and found both quantitative and qualitative differences between them. Quantitatively the numbers of colonies of fungi increased with increasing acidity. However with regard to the number of species both extreme alkalinity and acidity caused reductions in numbers. There was generally a decrease in numbers of species and isolates with depth due to several reasons acting at each habitat. Qualitatively many fungi were not restricted to one site, however there did seem to be a difference between the flora from alkaline soils and acid soils. There were two groups of Mortierella species - some occurred in the alkaline soils and some others in the acid soils, Zygorhynchus species occurred mainly in the acid soils. Penicillium was the most important genus occurring mainly in the acid soils and near the surface, Cylindrocarpon and Fusarium species were common in all soils. Trichoderma viride was relatively common in occurrence, but with a preference for the acid soils. Valerie Nicholls (1956) examined the flora of chalk soils and found that there are some

species which may be expected to be isolated from chalk soils but are not exclusive to them and other species which are characteristic of chalk habitats mainly. Many of the commonly occurring species are similar to those isolated by Warcup (1951) on the alkaline soils with the exception of Penicillium thomii. Some of the species isolated have a restricted distribution occurring only in one of the soils sampled.

Another feature which was found to influence the soil population was the surface vegetation and the resultant soil. Bisby, James and Timonin (1935) extended their study of Manitoba soils to comparing the fungi isolated from differently covered soils. They found that there were some fungi which were associated with certain types of surface vegetation. Penicillium thomii and Trichoderma viride both occur more frequently in forest soils although the latter does occur in the other samples, cultivated soils are preferred by Penicillium terrestre, P. chrysogenum and especially Fusarium species. Other species occur in both types of soil mentioned, these are Phoma species, Coniothyrium species and Gliocladium species, which they call a true soil fungus. There were other species which were associated with the other soil types studied. Aspergillus species were not very common in any soil profile. Mortierella species were the most common of the Phycomycetes and were isolated from all types of soil. They found that there was an influence of soil type and horizon on the fungal population so giving a characteristic flora.

There have been other studies conducted with regard to the surface vegetation. McLennan and Ducker (1954) in their study of an Australian heathland, found that the numbers of fungi did not immediately decrease with depth but that the B horizon was comparable with the A horizon and this was found to correlate with the carbon content of

of the B layer. Qualitatively many fungi were isolated which were cosmopolitan in distribution but there was an abundance of Penicillia and the flora was dominated by Mortierella rammanniana. Thornton (1956) showed a similar pattern of results from his studies of heath soils in Britain, but Mortierella rammanniana was not so dominant and Trichoderma viride increased in occurrence. He compared heath and woodland soils and found that the change in vegetation, which alters the soil also results in an alteration of the soil mycoflora, although many species were common to both soils.

Sewell (1959) examined the fungal population of Calluna-heathland and root surfaces. He found that the flora on the surface of the Calluna roots was influenced by the soil horizon rather than the root indicating that the soil type is important, further, the fungi isolated were similar to those isolated from other heathland soils, particularly Trichoderma viride and Mortierella species.

Parkinson and Balasooriya (1967) reaffirm many of the earlier findings in their study of the vertical distribution of fungi in a Pine wood soil. They found that some fungi occurred in all horizons and others have a more restricted distribution. One of the factors influencing the occurrence of fungi was the pH which altered over a short distance and there was a corresponding change in the mycoflora. Pine needles which are the main supply of organic matter into the soil influence the fungal population so that it is similar to other pine wood soils, although the soils themselves may be markedly different. This would seem to be confirmation of the idea that each soil has its own characteristic fungal community as well as a cosmopolitan flora, which in this case is particularly influenced by the surface vegetation supplying certain nutrients. England and Rice (1957) studied the soil fungi in a tall grass Prairie and an abandoned field and found that the

field was richer in fungi although the organic content was less. Qualitatively there were some species which were common to both sites and others more common in one site only.

It would seem, therefore, that soil type and surface vegetation are important in the production of a particular mycoflora. Tresner Backus and Curtis (1954) studied the soil fungi in close association with the surface vegetation. They found that there was a quantitative and qualitative change in the soil flora as the surface vegetation altered. There was an increase in numbers from the pioneer stage to the climax. The changing mycoflora may be influenced also by the moisture content and the organic content. The latter is shown by the change from sugar fungi to the decomposers of more complex substances being produced in the later stages of the succession. From these investigations it can be seen that the surface vegetation influences the soil population of established soils undergoing slow change, but what happens in soils which are lacking in an established surface vegetation such as spoil heaps, where the fungi are in direct contact with the soil without the influence of vegetation?

There have been several investigations concerning pioneer colonisation of soils of various kinds by fungi. One of the earliest studies was conducted by Bayliss Elliott (1930) on the salt marshes of the River Dovey. From samples taken low down on the marshes there were very few isolates probably due to the very adverse conditions. Most of the isolates came from the samples which were taken further up shore in the vegetated area. Although conditions are not very favourable for growth she concluded from her results that the fungi were active in association with organic matter as they would be isolated from organic debris even in the inhospitable muds.

Since that investigation there have been several studies to

ascertain whether fungi are active colonisers of virgin soils. Juliet Brown (1958) studied fungi in relation to sand dunes which show marked differences in development over short distances, in addition she studied two kinds of sand dune systems, one was alkaline and the other acid. Over a limited area the sand dune can change from a pioneer phase to a fairly stable environment with an equivalent development of the surface vegetation. Brown found that pH had a marked effect giving rise to distinct fungal communities in the two dune systems, with some species occurring in both systems. Some fungi have been found in sand dune soils with a similar reaction in other parts of the country so agreeing with the idea of a characteristic flora of certain soils. There was only sporadic occurrence of fungi in the open sand with many soil plates remaining sterile. Generally she found that the fungi which did occur were associated with extraneous organic material. There was one Phycomycete isolated from open sand and a variety of Fungi Imperfecti. Several Penicillia were isolated from both systems in the open sand - Penicillium restrictum, P. brevi-compactum, P. spinulosum, P. cyclopium, P. nigricans, P. terlikowskii, P. adametzi. There was also little fungal colonisation in the moving fore dunes which are subject to much variation. However, there was the development of a characteristic flora associated with this environment, with some species occurring regularly, notably Tilachlidium and Pyrenochaeta. There was a wider range of Penicillia isolated and more Fungi Imperfecti in comparison with the open sand. Gradually the mycoflora alters as the succession is continued from the pioneer stage. In the later stages there is a mycoflora which can be associated with the dune flora as there has been for other vegetational patterns. She also found that moisture was important. In the later stages the fungal flora compares with similar established sites in many respects, although some differences may be due to the younger

state of development. Brown also mentions that fungi may be important in stabilising the dunes by aggregating the sand particles.

A similar study was conducted by Pugh (1962) on a developing salt marsh. Pugh's results are in agreement with the previous work on soil populations suggesting that there is a characteristic flora associated with salt marshes. The numbers are lower than in normal soils probably because of the alkalinity of salt marshes and the regular flooding to which parts are subjected. There is a general upshore increase associated with the increased amounts of organic matter and less flooding. He suggests that the upshore increase of fungi in fact reflects the normal inhabitants of the developed marsh gradually colonising the lower areas as they become suitable, Cephalosporium acremonium is particularly evident in this connection. Other fungi such as species of Aspergillus, Mucor, Penicillium and Trichoderma viride increase downshore which is probably due to their introduction from outside sources and remain asspores or develop on organic debris. Also some of these fungi can tolerate the anaerobic conditions which prevail. This suggests that certain fungi are actual colonisers rather than merely following the 'higher' plants as fungi were sometimes isolated from areas of incomplete plant cover. The fungi isolated lower down on the shore are not considered to be true salt marsh inhabitants but transients as they are unable to survive in large numbers higher up the salt marsh. He concludes that the salt marsh is supplied with inoculum from several sources from which fungal colonisation may develop.

It would seem that fungal colonisation of unpopulated soils is limited until the arrival of other plant colonisers which provide a source of nutrients for fungal development, however, if there is some organic debris fungi can grow and develop in the most adverse conditions. Those fungi which are the primary colonisers are generally lost rapidly

as the community develops due to competition from other fungi (Pugh 1962). Also the fungal community develops and matures as the vegetation also adapts to its environment.

Bridge Cooke and Lawrence (1959) studied the development of the mycoflora of recently glaciated soils. One site was comparatively close to a vegetated area while the other was fairly distant from any vegetation. The mycoflora of both sites was influenced by the available organic material and nitrogen content which influenced both the numbers and species of fungi which were isolated. There was an increase in numbers once the surface vegetation became established and continued as it developed. The increase occurred at a later stage in the soil furthest from the vegetation and so further from a supply of inoculum for fungi and plants which is mainly wind-borne. Similar to Tresner et al. (1954) there was a corresponding change in the mycoflora as the surface vegetation changed and the organic materials altered. However, there were some isolations on the raw material before the surface vegetation became established. In addition to the development of the mycoflora with the surface vegetation there were also more fungi isolated at depth presumably due to nutrients being supplied by percolation.

Most of the fungal studies of soils have been carried out on naturally occurring environments. Disruption of the natural habitat by agriculture and its effect on the soil population has also been studied (Warcup 1957). However, there have been few studies on the unnatural environments created by man's industrial activity. There have been studies concerning reclamation and natural colonisation of industrial waste, in particular, coal spoil, but these have generally concerned the development of grasses and other plants and not the mycoflora of the waste (Richardson 1957, Hall 1957, Goodman 1965).

In a study of an artificial environment Price (1961) screened coal spoil for mesophilic fungi using a variety of isolation methods. Price found that the fungal population was qualitatively similar to that of the surrounding area but quantitatively inferior. He also suggested that although fungi may be able to live actively, as opposed to dormant survival, in a soil completely devoid of plants or plant remains, most activity occurred in soils that bear or have borne plant cover. It would seem though that fungi are active colonisers of coal spoil with or without the assistance of other plants.

Other studies of artificial environments, such as wood chip piles, industrial effluent have concerned thermotolerant and thermophilic fungi which are favoured by the higher temperatures which often occur. Evans (1969) studied coal spoil with regard to the thermophilic fungal population and found that in common with mesophilic fungi the size of the population was also influenced by the organic content of the spoil.

It would seem, from the studies of artificial and natural environments that fungi are not very efficient primary colonisers even in coal spoil which is not a poor substrate, but in the presence of organic debris they can survive very adverse conditions. Generally though they need some form of plant cover to act as a regular supplier of nutrients. It was decided to investigate lead contaminated soils, some of which were without plant cover, to examine whether fungi could colonise such an adverse environment, without the ameliorating effect of surface vegetation.

In addition to the qualitative and quantitative studies of fungi inhabiting the various soils and different parts of those soils there have been other forms of investigation. One field has been concerned with the factors which influence and affect the fungal growth in soils,

this was highlighted by Coleman in 1916 in a wide ranging study of environmental factors. Waksman (1924) found that pH was a factor affecting fungal growth by comparing the effects of different fertilisers which alter the soil reaction when added to the soil. Later Parkinson and Balasooriya (1967) were able to study the effect of a naturally occurring change in pH over a short distance in the same soil. They agreed that there was a distinct change in the flora which was mainly qualitative and they confirmed this in laboratory experiments. Other features of the soil environment which have been studied are soil moisture which seems to exert its effect in the case of drought in particular, so that only fungi with resistant structures are able to survive. Soil aeration is important in that most fungi are aerobes, this is influenced by the amount of soil moisture present. Griffin has discussed some of the environmental factors and their effect on the soil mycoflora (1972). Another feature which is receiving study is the spatial distribution of fungi on various types of soil particles (Gams and Domsch 1969).

Another feature which is important in the soil is the food supply available to fungi, as they are heterotrophic and so are dependent on food materials produced by other organisms. Garrett (1951) studied the adaptation of some soil fungi in relation to their substrate in the soil, showing the particular adaptations which enable them to survive in the soil. Jensen (1931) also studied various influences in the soil including food supply. In his work he also examined their role in the decomposition of some compounds such as cellulose. Fungi are known to be important in the decomposition processes in the soil (Bridge Cooke 1958). On adding cellulose to a fungal population the numbers increase rapidly showing that they are actively using the cellulose.

These researches concerned with the factors affecting fungal growth are important in providing some possible explanations for the findings of soil investigations concerning the mycoflora. However, the soil is such a heterogeneous environment that the field has many viewpoints but they are not mutually exclusive (Chester 1948) and lead to a general picture of the soil mycoflora. Some of the main points are discussed and summarised by Gray and Williams (1971). Many of the problems facing the soil microbiologist have been discussed by Burges (1963) in particular the assessment of the activity of the soil population and the heterogeneity of the substrate. This latter point is particularly relevant to spoil heaps which may change markedly over a short distance.

It was decided to study another artificial environment which had resulted from Man's activities and to examine the mycoflora both quantitatively and qualitatively. The study was conducted over two years to examine seasonal variation and also for confirmation of results, the samples were taken at several depths to see if there was any effect. In addition to the paucity of the environment and the lack of surface vegetation except where man has intervened another problem was studied and that was the problem of lead contamination. This is interesting in view of the present state of man's contamination of the world to see whether such organisms as fungi can adapt to these unfavourable conditions, as it has been shown that some plants can.

CHAPTER 1

MATERIALS AND METHODS

1.1 Chemicals

All the chemicals used were of A-R grade. The amino acids used were biochemical grade (chromatographically homogeneous) supplied by British Drug Houses Ltd., Poole, Dorset. The standard solutions used in the Atomic Absorption spectrophotometer were made up from B.D.H. Lead Nitrate Standard solution for atomic absorption spectroscopy.

The agar media and liquid medium were supplied by Oxoid Ltd., London S.E.1.

1.2 Collection of Samples

The samples were collected in polypropylene collecting bottles which had been sterilised by autoclaving at 20 lbs/ins^2 for twenty minutes.

The soil samples were taken by digging a small pit and then clearing material moved by the spade with a sterile spatula. The collecting bottles were then pushed horizontally into the side of the pit at appropriate depths. Samples were taken at the lowest level first then progressing upwards, in order to avoid material being dislodged into the wrong level. For the top soil samples the overlying debris, if any, was first removed. All instruments were flamed in 95% alcohol before and after use.

The samples were transported to the laboratory and examined and plated the same day.

1.3 Measurement of pH

pH readings were taken in the laboratory on a Pye model 79 using the soil from the collecting bottles after some soil had been removed for plating. Two grams of the soil sampled were mixed with twenty-five millilitres of distilled water, agitated and left for thirty minutes. The pH was then measured. The pH meter was standardised before and after each sample using a buffer solution of pH4.

1.4 Measurement of soil moisture

Soil moisture was determined using the soil from the collecting bottles. One gram of each soil was weighed into a small crucible of known weight, oven dried at 105°C for forty-eight hours and then weighed. The loss of weight on drying was calculated and expressed as a percentage.

1.5 Measurement of organic content

The organic content of the soil was determined using the soil from the collecting bottles. The soil was first dried at 110°C and then one gram of soil was weighed in a crucible of known weight. This soil was then heated in a Muffle furnace at 375°C for 16 hours. After cooling the crucibles with the soil were weighed and the weight loss calculated. The organic content is then expressed as a percentage.

1.6 Measurement of Lead content of the soil

Samples were collected in polythene bags from the pit as a whole. They were oven dried at 105°C and ground using a Wardell roller crusher. They were then sieved through an 80 sieve mesh and if necessary final crushing was done by hand using a pestle and mortar. The sieved samples were then ignited in a Muffle furnace at 700°C for forty-eight hours to remove any organic material. After ignition two

methods of analysis were carried out:

(a) Acetic Acid Extraction (adapted from Ruhling and Tyler 1968)

One gram of ignited sample was mixed with twenty-five millilitres of glacial acetic acid (A-R) in a nickel crucible. The mixture was then heated over a water bath for five hours. This method is used to extract the more readily available lead, probably that which is important biologically. After five hours the mixture was filtered and made up to one hundred millilitres with distilled water.

(b) Hydrofluoric/hydrochloric acid Extraction

(This is a modified method based on that devised by Pawluk, 1967).

One gram of ignited sample was weighed into a nickel crucible. Ten millilitres of concentrated hydrofluoric acid (48%) and ten millilitres concentrated hydrochloric acid were added and evaporated to dryness on a sand bath. This process was repeated. Five millilitres of concentrated hydrochloric acid were added to the residue and this mixture evaporated to dryness. Ten millilitres of 10M hydrochloric acid were then added and the solution poured into a volumetric flask and made up to two hundred millilitres using distilled water.

The solutions from both analyses were transferred to plastic storage bottles. The amount of lead in the solutions were measured using an EEL 240 atomic absorption spectrophotometer. (Elwell and Gidley, 1961, EEL handbook).

1.7 Methods of Isolation

The principal method of isolation used was that described by Warcup (1950) whose soil plate method was chosen because of its simplicity which enabled large numbers of samples to be dealt with

easily. After preliminary investigations it was decided that fifty milligrams of sample per plate was suitable for Sites 1, 2, 3 and 4. For Site 5, the agricultural field, ten milligrams was found to be sufficient to give a reasonable number of colonies. The soil was weighed in a sterile container and transferred to a sterile plastic petri dish. Cooled molten agar was poured into the plate and gently swirled separating the soil particles and spreading them around the plate.

Rose bengal at a concentration of 1:15,000 (Smith and Dawson 1944) was added in order to control not only the degree of bacterial contamination but also the rate of growth of fast spreading fungi.

The soil plates were incubated at 25^{o}C and examined at frequent intervals.

Potato Dextrose agar was found to be the most useful isolating medium, as compared with alternative media it was least selective. The alternative media Malt Extract agar, Cornmeal agar, Czapek Dox agar, Tap Water agar, were used in subculturing for identification purposes.

Two sets of plates were made up for each sample. One set contained potato dextrose agar only, the other contained potato dextrose agar with Lead Nitrate added at a concentration of 400 p.p.m. The lead nitrate solution was added to the molten agar and gently swirled to facilitate mixing. The problems of adding lead to solid media will be discussed more fully later (Chapter 8). Two plates of each type were made from each sample.

1.8 Subculturing

The plates were examined for colony growth daily, using a stereoscopic dissecting microscope. At first colonies were isolated using an inoculating needle but this did not prevent the plate being overrun by fast spreading species and leading to an exaggerated number of isolates of the same species from the same source. To avoid this problem it was decided that colonies should be isolated by cutting a piece of agar with the colony and removing it entirely from the plate. The piece of agar with the colony was placed on a new plate of agar. If a plate became overrun by a spreading colony then a "streak plate" was made. This means that the desired colony along with possible contaminants is streaked across an agar plate. If the plate is examined after twenty-four hours individual colonies can be picked off.

Isolates from the soil plates containing lead nitrate were transferred to plates without lead. This was done because it was found that the lead sometimes altered the character of some species so hindering identification. The cultures were then incubated at 25^{o}C for varying times depending on their rate of growth.

Permanent cultures (sub-cultured every six months) were stored in 1 oz McCartney bottles on potato dextrose agar.

1.9 Black Light

In addition to the use of various media for identification, a 'black light' bench was used. The 'black-light' bench was of the type described in Plant Pathologists Pocketbook (1968), and was used to induce non-sporulating isolates to sporulate.

1.10 Examination of Cultures

Cultures could be examined in the plates using a stereoscopic dissecting microscope or the low power objective (x10) of a Vickers M15c microscope. For a closer examination slides were made using Lactophenol or Lactophenol and Cotton Blue mixture.

1.11 Types of Experiment

(a) Solid Culture

Some experiments were carried out using Potato Dextrose agar in petri dishes. The agar solution was made up and autoclaved. The lead solutions were made up and autoclaved separately. The lead was added to the agar just before pouring, to give a known concentration of lead per plate. The agar was made up individually for each plate to try to ensure even distribution of lead in the medium.

(b) Liquid Culture

The culture medium used in the experiments was Oxoid Czapek Dox Liquid Medium (modified). However, if the constituents were to be varied the solution was made up using the individual constituents which were:- per litre of distilled water

Sodium nitrate	2.0 grams
Potassium chloride	0.5 grams
Magnesium glycerophosphate	0.5 grams
Ferrous sulphate	0.01 grams
Potassium sulphate	0.35 grams
Sucrose	30.0 grams

Details of any additions or changes in the basal medium will be given in the appropriate experimental section. The pH of the medium was adjusted using N10 hydrochloric acid.

Media were usually sterilised by autoclaving at 10 lbs/ins^2 for twenty minutes. However if there were substances added which are damaged in some way by autoclaving then the medium was sterilised using a Millipore filter with 0.2μ diameter pores.

Inocula for the experiments were grown on potato dextrose agar sloped in 8 oz Medical Flats. Spore suspensions were obtained by adding ice-cold sterile distilled water to the culture bottles and agitating. The suspension was then diluted, if necessary, using distilled water to a concentration of 1.5 - 2.0 x 10^6 spores/ml. Haemocytometer counts were used to ascertain the concentrations of spores.

The experiments using liquid medium were carried out in still culture. The flasks were inoculated and placed in a constant temperature room and left until the time for measuring the amount of growth.

1.12 Measurement of Growth

(a) Solid Culture

In these experiments the growth was determined by measuring the diameter of the mould colony. Two axes were measured usually the shortest and the longest diameters and the results expressed as the mean.

(b) Liquid Culture

Growth was measured by ascertaining the dry weight of the mycelium. Whatman No. 5 filter papers were previously dried and weighed. The papers were than used to filter the liquid cultures, and mycelial

growth washed with distilled water. The filter paper, with the mycelial growth on, was then oven dried at 70°C for twenty-four hours and weighed after cooling to room temperature in a desiccator. The dry weight is expressed in milligrams.

1.13 Measurement of Sporulation

In some experiments the number of spores produced was measured. The mycelial mat was carefully removed from the Erlenmeyer flask and transferred to a 250 ml flat-bottomed flask containing 50 ml of 0.5% (v.v.) aqueous solution of 'Teepol'. The flask was shaken for fifteen minutes at high speed on a Griffin wrist action shaker. The solutions were made up to 100 ml and the number of spores per ml was calculated using a haemocytometer slide. In some cases the spore concentration was so great that further dilutions were made. An average of three counts was taken as the final value. The results are expressed as spores/ml.

1.14 Replication of Results

Experiments were carried out using replicates of four. The mean of the four flasks/plates was then taken as the result. Sometimes more replicates were used or the results of separate but identical experiments are amalgamated to give the final results.

1.15 Hanging Drop

Spore suspensions were made up as for liquid inoculation varying the solution according to the needs of the experiment. A Van Tiegham ring was vaselined on both sides and one side attached to a normal microscope glass slide. A drop of spore suspension was placed on a clean glass coverslip, and the coverslip and drop inverted maintaining the drop in the centre of the coverslip. The coverslip

was placed on the Van Tiegham ring ensuring that the drop did not touch the vaseline or the sides of the ring. The drop was therefore in an air tight chamber, and could be examined under the microscope and counts of germination made.

1.16 Organisms

Some species used in the experimental investigations were isolated from the lead spoil heaps under investigation. All other cultures were obtained from the Commonwealth Mycological Institute.

Species used from the lead mine spoil heaps

(numbers are those assigned by C.M.I. when sent for an identification check)

Absidia coerulea Bain.	I.M.I. 155754
Aspergillus fumigatus Fres.	I.M.I. 152482
Cephalosporium acremonium Corda.	I.M.I. 158396
Cylindrocarpon olidum Wollenw.	I.M.I. 148788
Fusarium sambucinum var. caeruleum Wollenw.	I.M.I. 160850
Gliocladium roseum Bain.	I.M.I. 151137
Penicillium frequentans Westling.	I.M.I. 147427
Penicillium simplicissimum (Oud) Thom.	I.M.I. 152489
Phoma herbarum Westend.	I.M.I. 155755
Trichoderma viride Pers ex Fr.	I.M.I. 152488
Verticillium psalliatae Treschow	I.M.I. 163634

Species obtained from the Commonwealth Mycological Institute

(With catalogue numbers)

Absidia coerulea Bain.	I.M.I. 38501
Aspergillus fumigatus Fres	I.M.I. 16062
Aspergillus fumigatus Fres	I.M.I. 28646
Aspergillus fumigatus Fres	I.M.I. 35570
Aspergillus fumigatus Fres	I.M.I. 45338
Aspergillus fumigatus Fres	I.M.I. 69714
Aspergillus fumigatus Fres	I.M.I. 89353
Aspergillus fumigatus Fres	I.M.I. 89354
Aspergillus fumigatus Fres	I.M.I. 94164
Aspergillus fumigatus Fres	I.M.I. 96202
Aspergillus fumigatus Fres	I.M.I. 108008
Aspergillus fumigatus Fres	I.M.I. 121660
Cephalosporium acremonium Corda	I.M.I. 96201
Cylindrocarpon olidum Wollenw.	I.M.I. 73501
Fusarium sambucinum var. caeruleum Wollenw.	I.M.I. 96282
Gliocladium roseum Bain.	I.M.I. 101020 (h)
Penicillium frequentans Westl.	I.M.I. 92265
Penicillium simplicissimum (Oud) Thom.	I.M.I. 61388
Trichoderma viride Pers ex Fr.	I.M.I. 24039
Verticillium psalliotae Treschow	I.M.I. 90861

One culture was obtained from the Centraalbureau voor Schimmelcultures, Phoma herbarum Westend. C.B.S. 36861

SECTION I

ECOLOGICAL STUDIES

been recolonised by grasses Mill Close is too recent for natural colonisation to have progressed much.

Soil samples were taken from five sites, four of which are man made spoil heaps and the fifth an agricultural field which has been contaminated with lead. Each site will be described individually later.

2.2 Preliminary Study

A study was carried out to discover the number of samples necessary to obtain an accurate representation of the species occurring in the soil at these sites. This study was carried out on the topsoil since it is likely that this fraction will contain the largest number of species. Ten samples of soil were taken from two of the sites (2 and 5) and five samples were taken from the remaining three sites. The procedures followed were described in Chapter 1. From each soil sample four soil plates were made, the soil being selected at random.

The results are presented in Tables 1 - 5 which lists the species isolated from each sample at each site. The results are also presented graphically in Figs. 2.1 - 2.5. The graphs show the cumulative number of different species isolated from the soil samples.

Tables 6 - 10 show the number of different species isolated on each of the soil plates from each sample. These show that four soil plates are generally sufficient to form an accurate sample.

Five samples were taken from Site 1 and from the graph (Fig. 2.1) it can be seen that this number was insufficient to isolate all the species from this site.

In Site 2 (Fig. 2.2) eight samples were enough to isolate all the species present, the remaining two samples did not add any different species. Since Sites 1 and 2 are similar it is feasible to suggest that

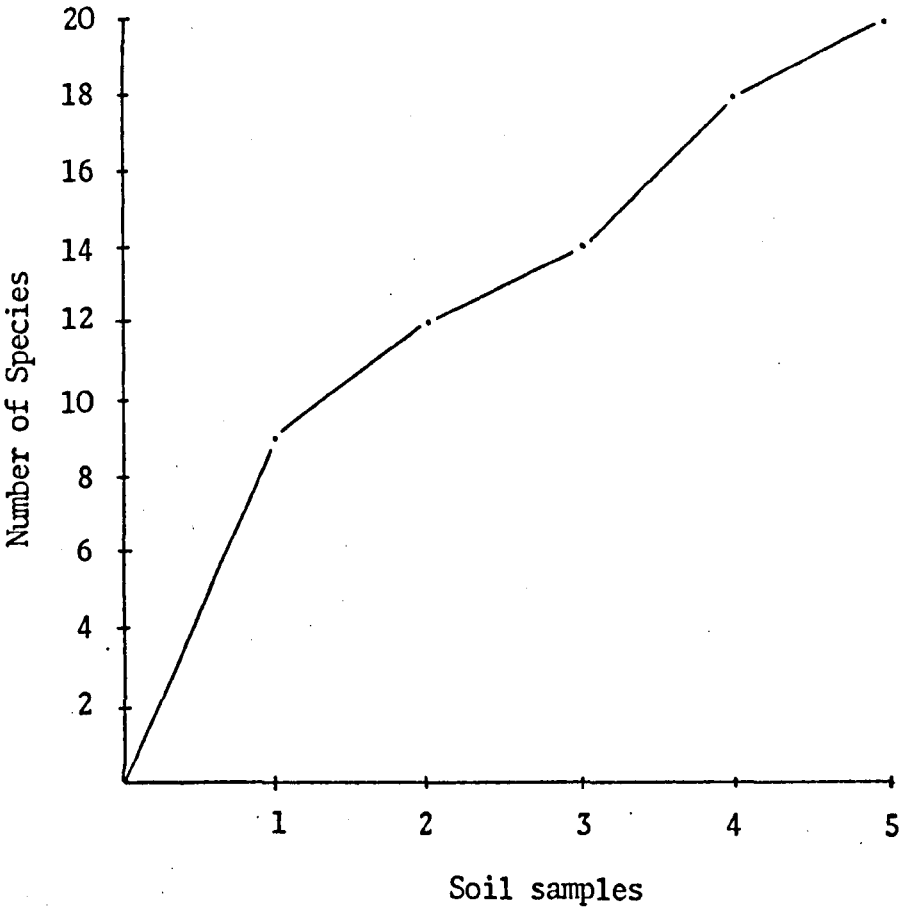


Figure 2.1

Number of different species isolated in each of five samples from Site 1

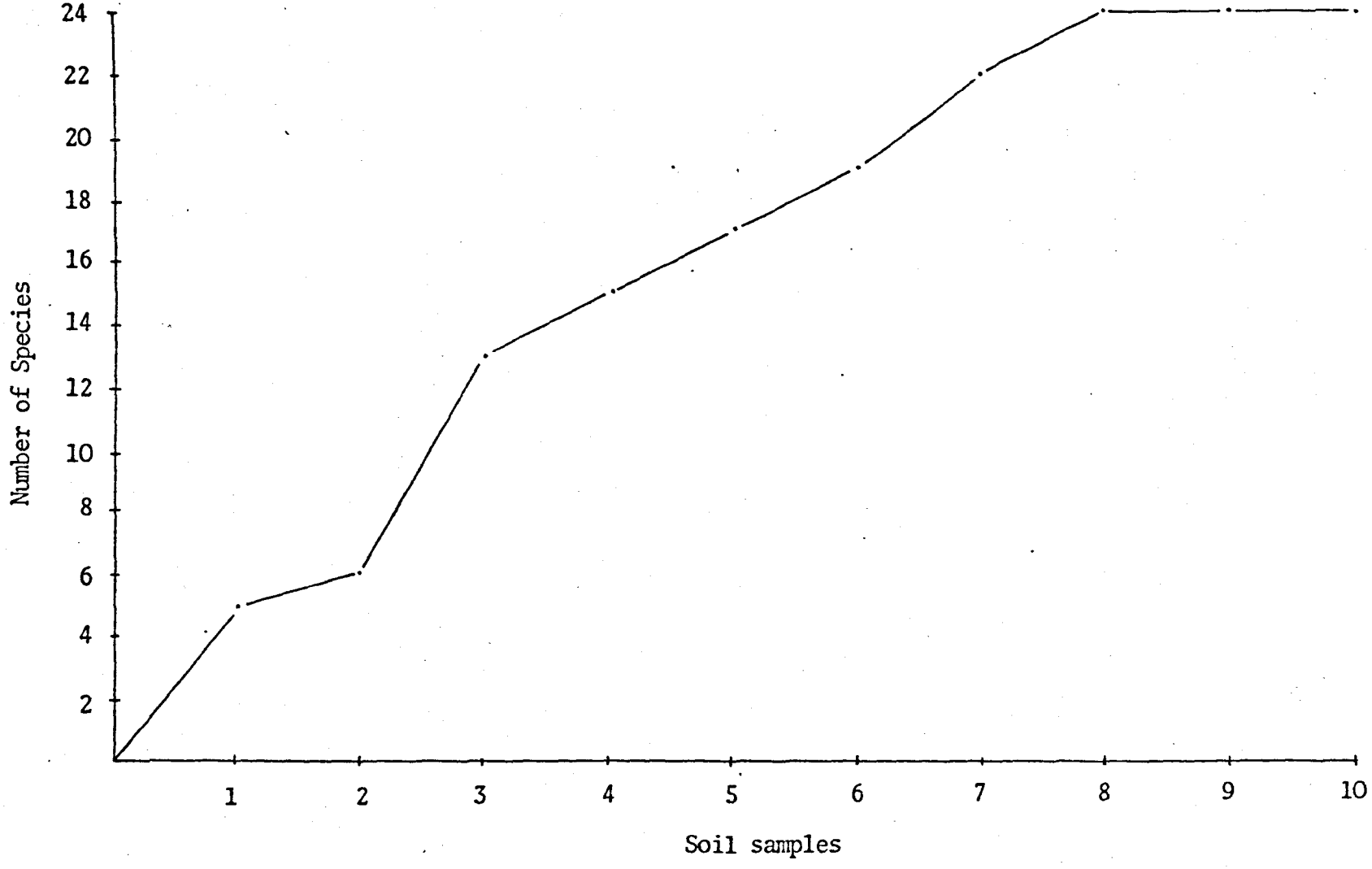


Figure 2.2

Number of different species isolated in each of ten samples from Site 2

a similar number of samples may be necessary to give a true picture of the mycoflora of Site 1.

Five samples were taken from Site 3 (Fig. 2.3) and there are no different species isolated after three samples, so that fewer samples are necessary to examine the mycoflora of this site than Sites 1 and 2.

At Site 4 (Fig. 2.4) also there were only five samples taken, however the number is not sufficient to isolate all the species present in the soil since no two samples contain the same species. However there is a decrease in the numbers of different species isolated after the third sample.

Site 5 (Fig. 2.5) contains more species than the other sites, however after nine samples the different species were isolated. It must be remembered that the results for this site came from 10mg of soil as opposed to 50mg from the other sites. This indicates the variety and numbers from Site 5.

From these results it can be seen that in order to obtain a complete picture of the mycoflora of these sites an extremely large number of samples would be necessary, which would be increased if depth were considered.

In addition to examining the numbers of species isolated the samples taken in this study were analysed for organic content. The method used was described in Chapter 1. Loss of weight on ignition is generally believed to give an indication of the amount of organic matter present rather than a definitive figure and as such is only useful as a comparison between the five sites investigated. The amounts are expressed as a percentage and are made up of the results of five individual samples which have been averaged and are presented below:-

Site 1 - 7%
Site 2 - 8%
Site 3 - 7%

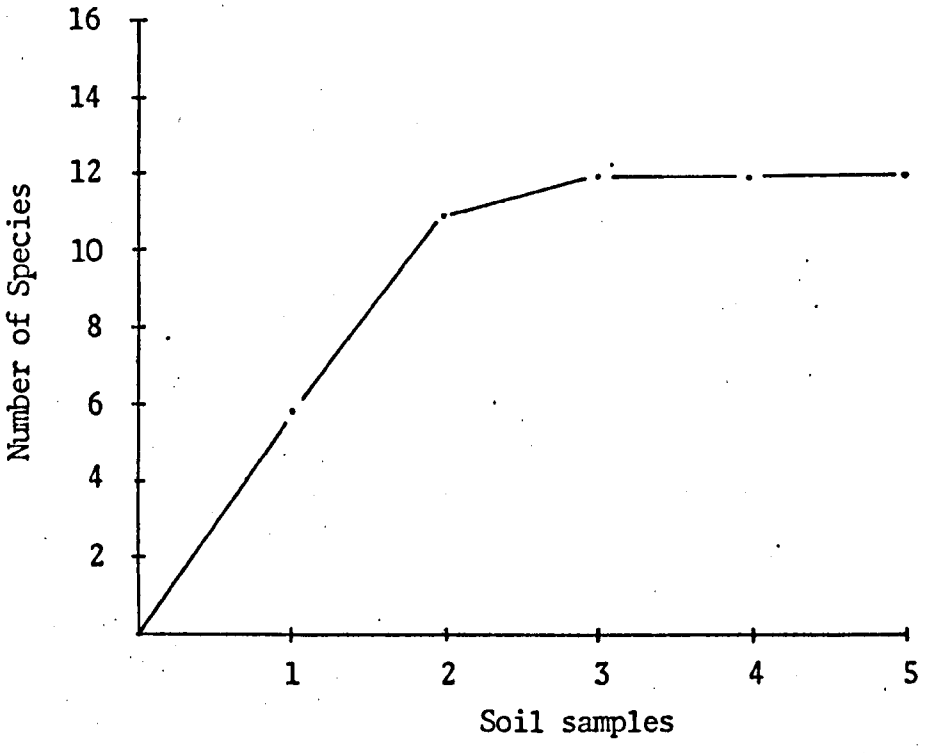


Figure 2.3

Number of different species isolated in each of five samples from Site 3

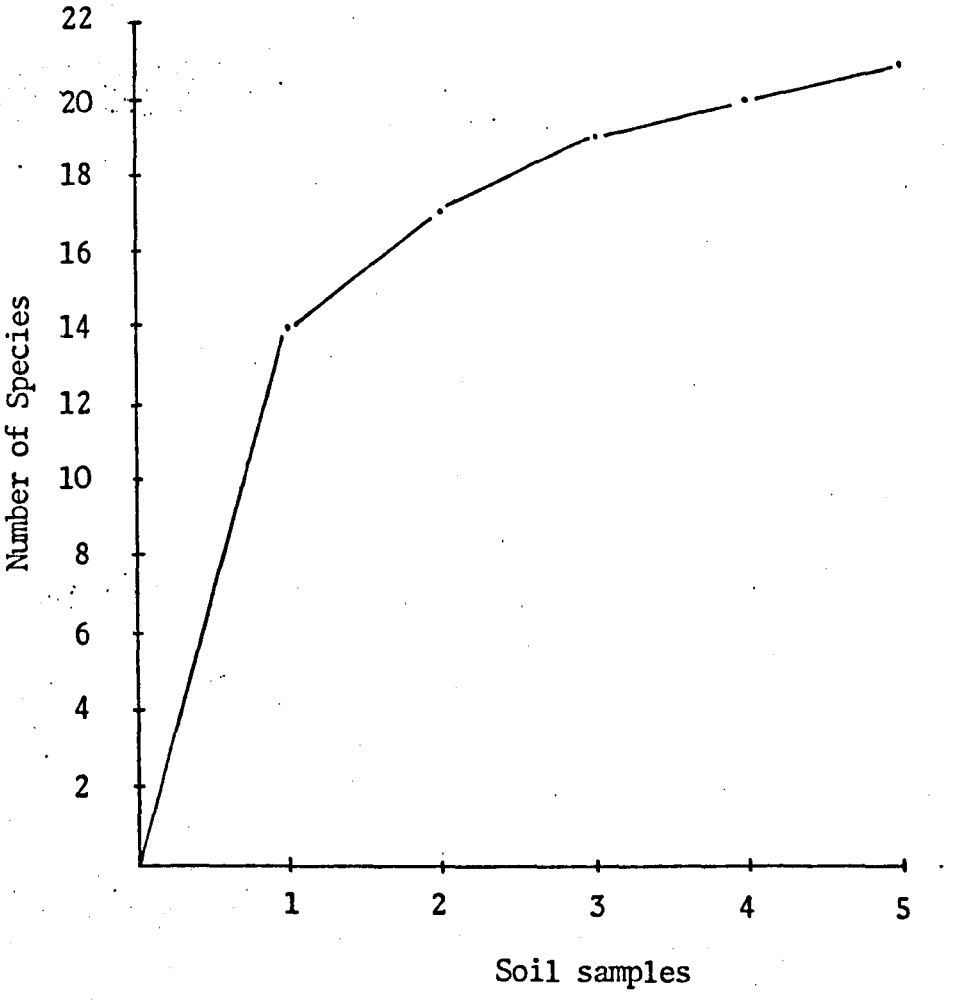


Figure 2.4

Number of different species isolated in each of five samples from Site 4

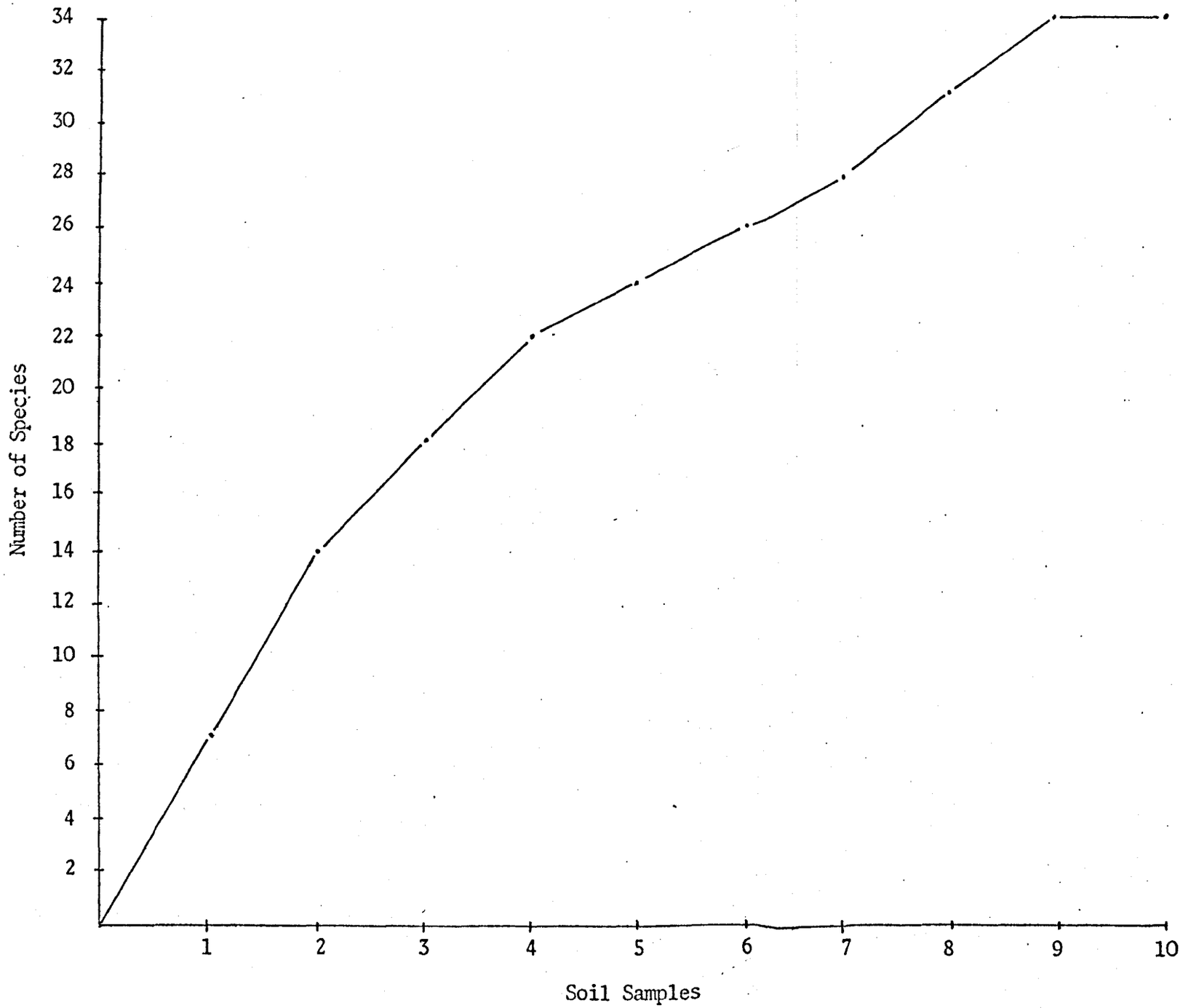


Figure 2.5

Number of different species isolated from each of ten samples from Site 5

Site 4 - 13%

Site 5 - 18%

Since colonisation of soils occurs first in the topsoil and then progresses downwards (Bridge Cooke & Lawrence 1959), the topsoils probably contain the most organic matter in the profiles.

The reason for the relatively high percentage at Site 4, when compared with the other spoil sites, is the fact that topsoil was added in December 1970. This certainly introduced much organic material which would not otherwise have been present. Site 5 contains the most organic matter as would be expected since it is an agricultural field undergoing a normal regime which results in some organic matter being present. Sites 1, 2 and 3 are relatively poor as would be expected since they are composed of industrial waste and have little surface vegetation, the only contributor to the organic content probably being the microflora.

2.3 Sampling and presentation of results

Soil samples were taken at several depths from the five sites. One sample was taken every 2 months. Two of the soil plates contained lead and two were without lead. This addition of lead to half the soil plates influenced the results. At all the sites there were fewer isolates from the lead containing plates. The results are expressed as percentage frequency of isolation and percentage occurrence. The results are arrived at by the following formulae:-

$$\% \text{ frequency of isolation } \frac{\text{Total no. of isolations of a given species}}{\text{Total no. of isolations of all species}}$$

$$\% \text{ occurrence } \frac{\text{No. of plates on which a given species occurs}}{\text{Total no. of plates.}}$$

On all sites the results for March 1971 were affected by failure of the incubator heating system so that the soil plates were subjected to temperatures around $0^{o}C$ at the beginning of incubation.

The results of the individual samples have been combined to form four groups of three samples. The samples taken in May, July and September are designated Summer and those in November, January and March as Winter. The numbers of isolates have been presented as isolates/gram so that direct comparisons can be made with Site 5 and other studies where the amount of soil used may be different. By combining the results in this manner there is some loss of detail however it was the only means whereby sufficient samples could be studied by one person to produce meaningful results.

2.4 Seasonal Variation and Profile Studies from Site 1

2.4.1. Description

This spoil heap, part of which is still in use, has not accumulated as a direct result of mining operations but owes its existence to the ore processing necessary for marketing. The waste under investigation was produced many years ago when the lead ore, galena, was smelted in open hearth furnaces - known as Newnham Hearths - to produce metallic lead. The sulphur from the ore was liberated to form sulphur dioxide and sulphur trioxide in the furnace gases. The furnace gases were then filtered to try to eliminate the lead dust, and passed through a wooden tower where they came in contact with a lime solution. The furnace gases were cleared of sulphur dioxide and sulphur trioxide which formed calcium sulphate and calcium sulphite in the sludge which also contained some of the lead dust. The sludge was pumped out into dams and left for the water to drain away. Thus the basic content of this spoil heap is calcium sulphate, calcium sulphite and calcium oxide.

Site 1 is situated at the edge of this spoil heap, which is a very crumbly area. The spoil consists of a friable base with a variety of small stones and pebbles at the surface (Plate 1b) which is grey in colour

(a) General view of Sites 1 and 2 showing nearby scrubland (July 1971)

PLATE 1

(b) Close up view of Sites 1 and 2 with Site 1 on the left (July 1971)

but at approximately 12cm depth the spoil changes colour and becomes fawn and sandy in texture. There is very little vegetation on the spoil heap, just a few clumps of grass which are not found near the edges (Plate 1a). The apoil heap is adjacent to an area of scrubby land which is a possible source of wind-borne seed or spores.

2.4.2. Results and Discussion

The results of sampling are presented in Tables 11 - 22 in the Appendix. The general pattern of the fungal population is presented in Figure 2.6 which shows the numbers of isolates/gram and the number of species. These figures show a similar pattern during the two years indicating that the changes are not simply an increase or decrease in growth but a variation in the numbers of species. There seems to be no regular seasonal variation, in fact, with the exception of the summer of 1971 the numbers vary very little indicating that the conditions affecting the mycoflora are probably very stable. Generally it is considered that surface vegetation affects the seasonal pattern of the soil mycoflora by influencing the organic content of the soil and as there is no surface vegetation in this site it would seem that the major influence on the mycoflora has been removed so that no seasonal variation would be expected. The increased activity in summer 1972 which is not only more growth of existing species but also the appearance of additional ones is not very marked but probably indicates slightly more favourable conditions allowing a wider range of species to develop in this site. However in the winter of 1972-73 the conditions have possibly reverted to those occurring in the two seasons prior to the summer 1972 so that the numbers of species is similar, the increase has been lost. There is a more marked reaction in the numbers of isolates indicating a decline in the amount of growth. The numbers of isolates

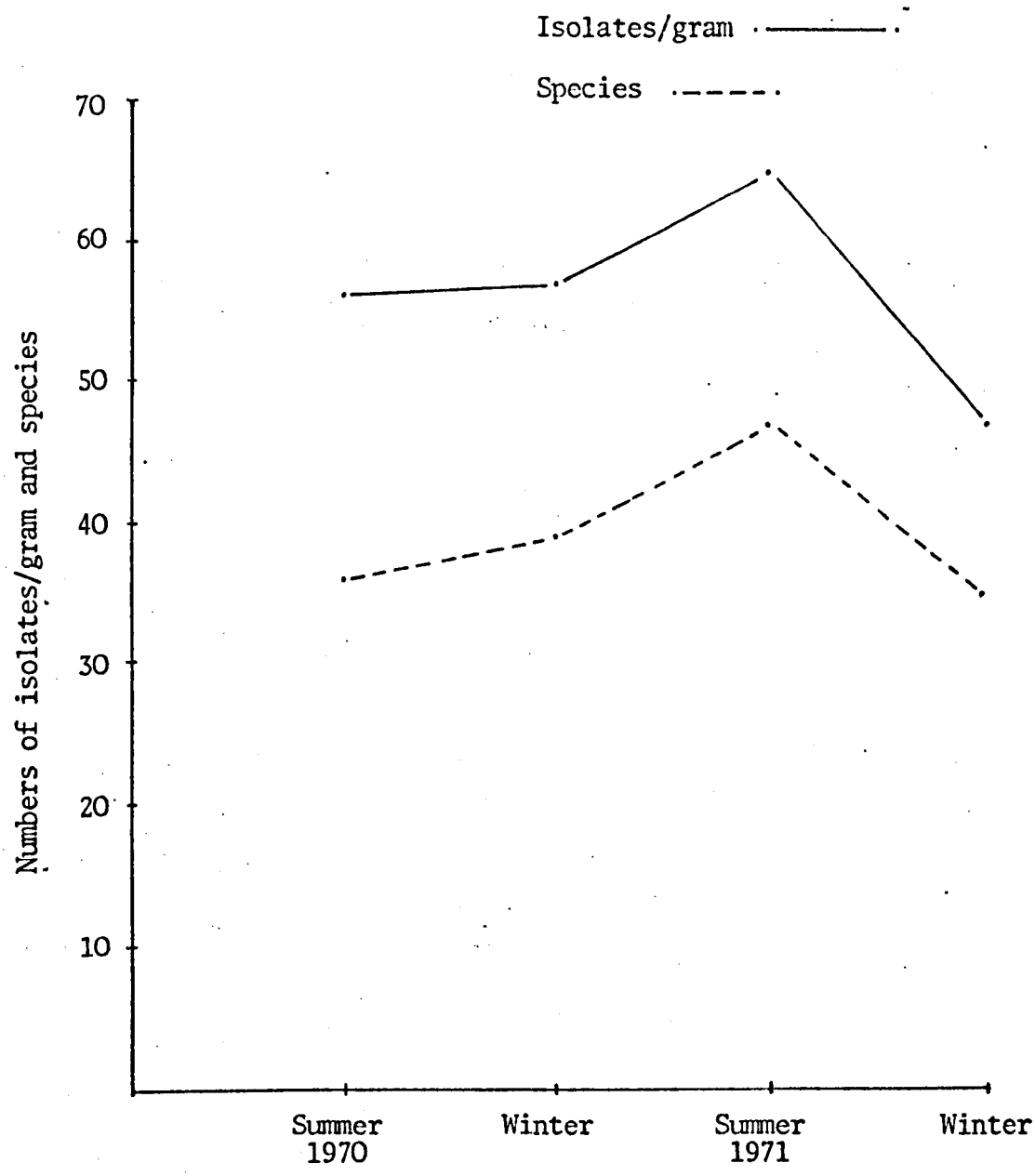


Figure 2.6

Number of isolates/gram and species isolated from Site 1 during two years

occurring at any time during sampling is markedly low in comparison with normal soils which generally contain fungi in hundreds of thousands (Burges 1958).

It was decided to analyse the two variables being studied - species and isolates - to see if there was any correlation between them. The regression line was selected as it shows ranges of both positive and negative correlation between two variables and can be presented graphically. It is not just the gross difference that is registered in the statistical analysis but whether there is any relationship between the two variables. As growth occurs the numbers of isolates of each species increases and varies so that the correlation is low. In a soil study of this kind using the soil plate method the correlation coefficient may show the situation in the soil, but it may also be a result of the fact that certain types of fungi are favoured by this method, particularly heavily sporing fungi and fast growing forms. These types of fungi would possibly influence the correlation so that it would be lower than it might otherwise be. The results must therefore be studied with this factor in mind. The results of the individual samples have been used for the analysis because the species and isolates are actual comparable figures also it gives a greater number of points to work with.

The analysis confirms that there is a statistically significant relationship between the two variables (Fig. 2.7). The closeness of the relationship, significant at the 0.1% level, implies that there is some growth but not sufficient to interfere with the relationship of the numbers of isolates and species. This means that any fluctuations during the two years occur in both numbers of isolates and species and that at no time is there exuberant growth of any species which would markedly increase the number of isolates without increasing the number of species.

Graphical analyses of the effect of season and depth on the fungal

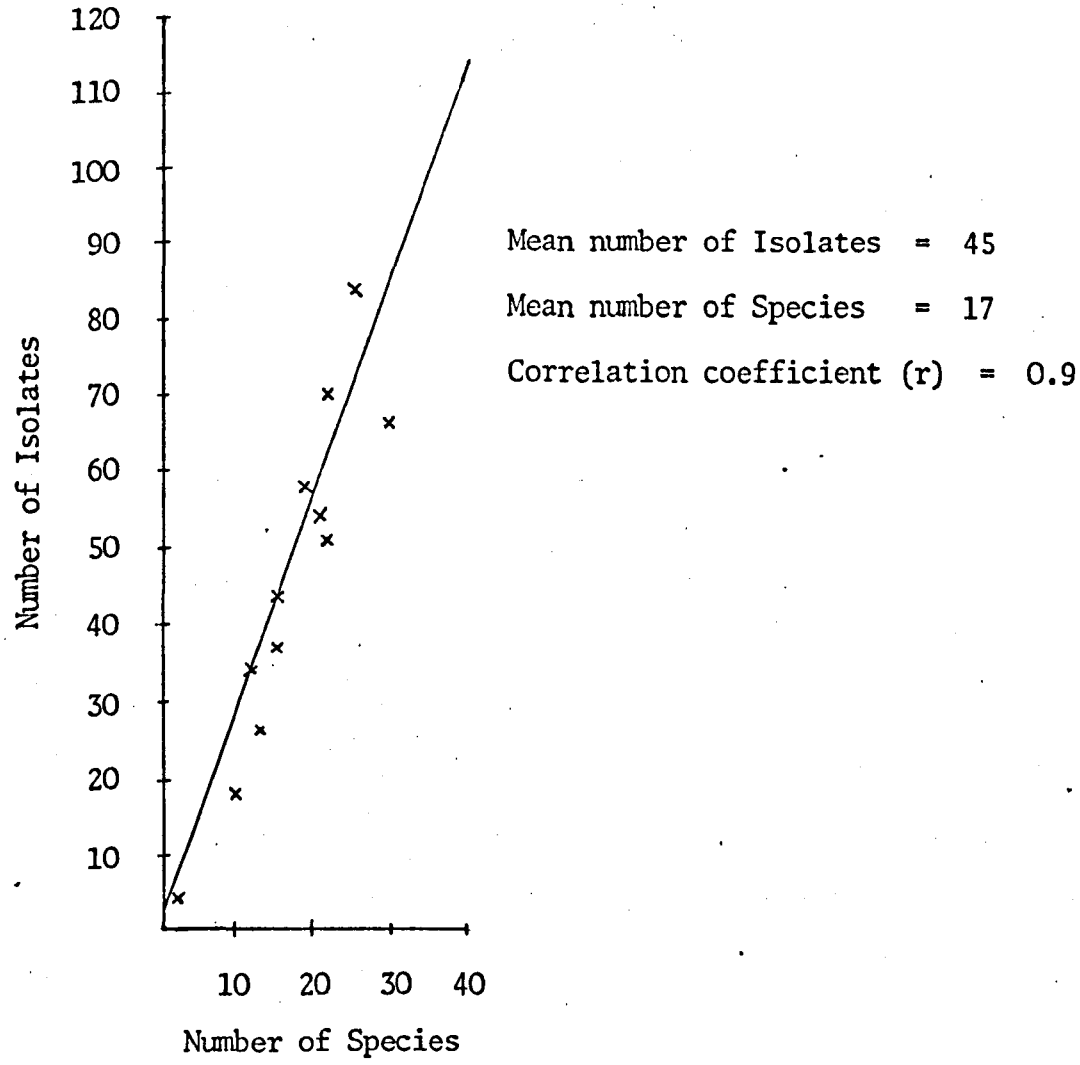


Figure 2.7

Regression line of the number of isolates on the number of species from Site 1

population are presented in Fig. 2.8 - 2.9. The numbers of species at different depths (Fig. 2.8) generally show a decrease with increasing depth, the numbers generally being highest in the topsoil fraction and lowest at 25cm depth. The numbers in the topsoil and 25cm fractions show a similar pattern to the total numbers of species although the variation is greater in the topsoil. This is to be expected since this fraction is more open to external influences than the 25cm fraction. At 5cm depth there is an increase in the second season which is maintained in the following season and then a decline. Whereas at 15cm depth there is a gradual decline in numbers during the two year period. It is difficult to explain these variations, it would seem that other factors are affecting these horizons particularly in summer 1972 which seems to be favourable in the topsoil and 25cm samples increasing the numbers but not in the other two samples.

The numbers of isolates/gram at different depths (Fig. 2.9) also show a decrease with increasing depth with the topsoil generally contributing most isolations and the 25cm samples the fewest. The pattern of the numbers of isolates over the two years is similar in these two levels. However there is much greater fluctuation in the topsoil probably due to external influences which are not so likely to occur at 25cm depth. At 25cm depth there is not much growth going on when the numbers of isolates are compared to the numbers of species. There is much more growth occurring in the topsoil which is a more favourable environment and so would be expected to foster more growth. The relative importance of the topsoil can be shown by the fact that the pattern of isolates is reflected by the numbers occurring in the topsoil. At 5cm depth there is very little fluctuation in numbers which perhaps reflects the stability of the environment which may be protected from external influences by the topsoil. At 15cm depth

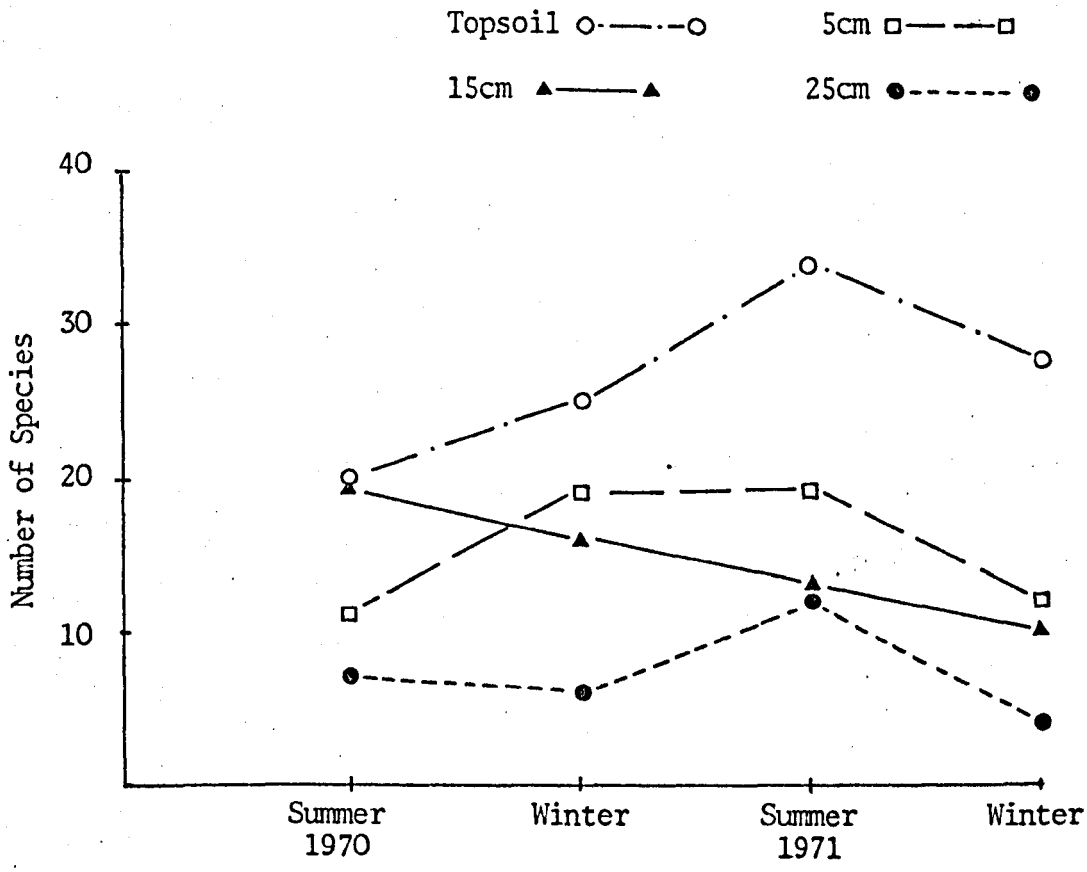


Figure 2.8

Number of species at different depths from Site 1

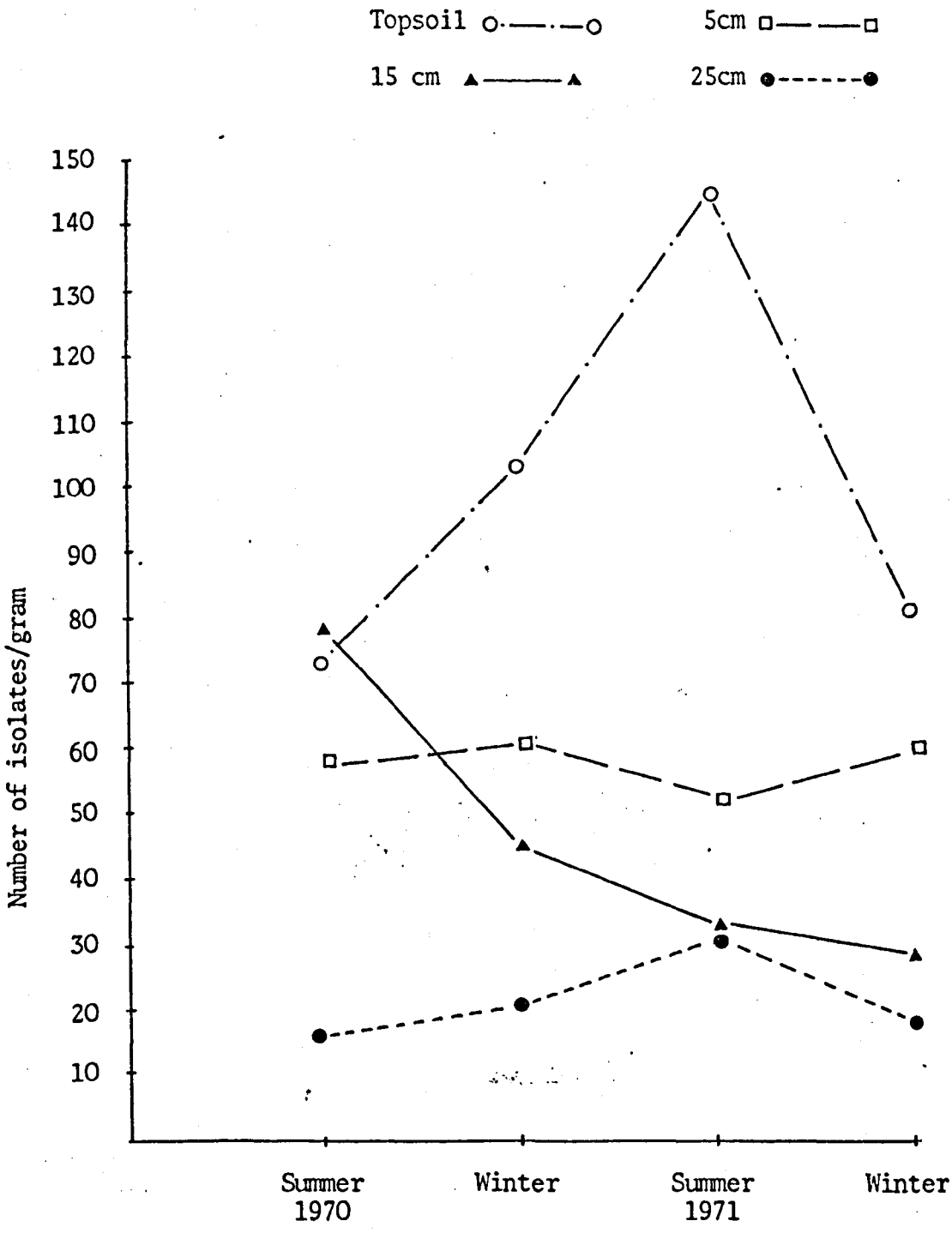


Figure 2.9

Number of isolates/gram at different depths from Site 1

the numbers gradually decline over the two years as did the numbers of species. This suggests that the environment at this depth is becoming increasingly hostile to growth of fungi. It is likely that there are either different influences acting at the various horizons or that the reaction of the mycoflora varies with depth. It is difficult to suggest what particular factors could be producing this varied reaction.

The range of pH, the average soil moisture and average lead content for the four seasons are presented in Table 2.1, as these may be of importance in influencing the fungal population. The individual results are presented in the Appendix Table 23. These can only act as indicators of the state of the spoil as the fungal habitat is on a much reduced scale. From the table it can be seen that there is little variation in the range of pH with depth or season. Generally the spoil is slightly alkaline. Since there is little variation it is unlikely that it is having any influence on the fungal population.

Another feature which may affect the soil population is the amount of moisture present in the soil. Soil moisture exerts its effect in many ways and is closely associated with texture, structure and air content of the soil (Griffin 1972). With regard to the mycoflora, soil moisture exerts its most direct effect through drought or waterlogging.

At this site the top two layers contain markedly less moisture than the lower two levels probably due to the coarse nature of the upper layers. Most moisture occurs at 25cm which may be due to drainage from the upper layers. There also seems to be a slight increase in soil moisture in the upper three layers in the winter months although the rainfall is fairly uniform throughout the year the evaporation from the soil would vary. There seems to be no regular pattern which would indicate some marked influence of soil moisture. The topsoil and 5cm depth contain similar

Table 2.1

Average lead content, soil moisture and range of pH of soil samples from Site 1

Samples	Lead content (in p.p.m.)		Topsoil		5cm		15cm		25cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
Summer 1970	5,333	10,520	7.7 - 8.6	3.1	7.1 - 8.6	3.4	7.4 - 8.1	14.6	6.4 - 8.3	32.0
Winter	10,083	17,933	6.9 - 7.8	6.6	6.9 - 7.5	6.0	6.7 - 8.4	17.7	6.6 - 7.9	22.0
Summer 1971	5,800	8,066	7.4 - 8.0	2.8	7.4 - 7.9	3.4	7.3 - 7.4	11.1	7.2 - 7.6	27.0
Winter	7,166	10,766	7.9 - 8.2	6.2	7.5 - 8.6	5.9	7.9 - 8.1	18.8	7.9 - 8.1	21.0

amounts of moisture and yet their numbers of isolates and species are not similar. It is likely therefore that their relative dryness does not hinder the development of the mycoflora. The constant high amount of moisture at 25cm may be a factor influencing the low numbers of isolates and species by decreasing the air and the pore space. This may also be acting to a lesser extent at 15cm although there is no increase in moisture over the two years to correspond with the gradual decrease in the numbers of isolates and species.

One of the main features of this study is the fact that the soils being studied have been contaminated with lead. The amount of lead in the soils has been measured using two different extraction procedures. Using acetic acid the lead removed is considered to be the easily extractable lead and this is the amount believed to be significant biologically. Hydrofluoric acid and hydrochloric acid mixture is supposed to remove all the lead so that comparing the two amounts one can see how much lead is tightly bound to the substrate. As would be expected the Hac extracted lead is less than the HF/HCl extracted, although the difference may vary.

Considering the amount of lead extracted by acetic acid the summer seasons contain similar amounts which are less than the winter seasons. It would be expected that this would lead to some variation in the numbers of isolates with increases in the summer samples as they contain less lead and corresponding decreases in winter. Only summer 1972 shows the appropriate reaction in the numbers of isolates and species. It would seem really that the lead does not have a direct influence on the fungal population but that it is a factor in the general lowering of numbers in conjunction with the total paucity of the environment.

From a qualitative aspect there was a variety of species isolated - 96 in all. Most of these species belong to the Fungi Imperfecti which

usually constitute a large proportion of airborne spores and so would be expected to occur in the topsoil. Some of these fungi may be trying to establish themselves at this site not merely appearing as contaminants.

In his studies on Calluna heathland Sewell (1959) suggests that the use of constancy of fungal isolates rather than frequency diminishes the advantage possessed by fungi with a high sporing capacity and fast growers on the soil plate. However, their constancy may be due to their ability to grow on soil plates. It was decided to use this method in any case to analyse the results of this investigation. The mycoflora is examined in two ways, first the constancy of the species is measured by its occurrence in the four seasons. Secondly the species are examined with regard to their appearance in the individual samples and are regarded as regularly occurring if they are found in six or more samples out of twelve. For a species to occur in the four seasons indicates its presence at all times although it does not indicate its ability to grow at all times but simply its persistence. However if a fungus occurs in large numbers then it may well be isolated regularly in the individual samples. Also a fungus may not occur in one season but occur in all the other individual samples indicating that it did grow strongly but the habitat was not suitable at that time. In conjunction these two analyses give a fairly accurate picture of the major members of the mycoflora.

From Table 2.2 it can be seen that most of the species isolated

Table 2.2

The number of seasons in which a species occurs

1 season	- 61 species
2 seasons	- 19 species
3 seasons	- 7 species
4 seasons	- 9 species

occur in only one season. In fact 83% of the species isolated occur in two seasons or less. These fungi constitute the occasional flora, many of

which occurred only in the topsoil and were probably airborne and not really members of the mycoflora of soils. Others may be trying to establish themselves in the soil and possibly occur in such low numbers that they are only occasionally isolated or they may be killed off while trying to become established by a hostile change in conditions or just the hostility of the environment.

The species which occur in all four seasons are:- Aspergillus fumigatus (75%), Aspergillus versicolor (33%), Coniothyrium fuckelii (50%), Epicoccum purpurascens (50%), Penicillium frequentans (33%), Penicillium funiculosum (58%), Phoma herbarum (83%), Trichoderma viride (58%) and sterile mycelia (100%). The figures in brackets refer to the percentage of individual samples in which the fungus occurs. All these fungi are normal soil inhabitants having been isolated in other soil investigations. The majority of them occur in large numbers most of the time. A. versicolor and P. frequentans it seems, occur regularly but in very low numbers since they only occur in four individual samples but in four seasons and only in a few isolates. This fungus may be a later invader of the habitat trying to establish itself or else it may be that the existance of a few hardy isolates is all that can be maintained. Excluding the sterile isolates, which make up a large number of inhabitants in many soils, Aspergillus fumigatus and Phoma herbarum were the most consistent species. There was no indication of seasonal variation in the numbers of isolates of these two species except for a slight increase in the summer months but nothing significant. Both fungi are considered regular members of soil flora, although P. herbarum is more prevalent on vegetable debris (Boerema 1964). In the remaining species occurring regularly Trichoderma viride tends to increase in activity in the late summer-early winter months. Coniothyrium fuckelii tends to occur more in autumn and winter.

Another method of evaluating the results is that of density of isolates, as used by Tresner, Backus and Curtis (1954). This analysis gives an impression of the constitution of the bulk of the mycelium in the soil as isolated by the soil plate method. The method of isolation will affect the types and frequencies of all fungi isolated, and so must always be borne in mind when considering the results. I have decided to consider only those species which constitute more than 5% of the total otherwise some infrequently occurring species would have to be included. Most but not all of the species involved occur regularly although some other species which do not occur regularly are considered.

There are only two species in addition to sterile isolates which contribute more than 5% of the total isolations. The other two species are Aspergillus fumigatus and Phoma herbarum. Trichoderma viride almost produces 5% of the total but the remaining species contribute very few as individuals. The difference is quite marked. It is usual that only a few fungi should contribute a large proportion of the total since Thornton (1956) found six species in a forest soil and four in a podzol contributing more than 5%. Thornton suggests that these species achieve dominance in undisturbed soils because of favourable conditions. In this soil it is more likely that these few fungi can tolerate the harsh conditions while the other fungi can hardly do so. Thus leading to the marked dominance of a very few species.

Most of the samples contain some isolates at the lower soil levels but the numbers are usually low, both individually and in total. Aspergillus fumigatus and sterile isolates were the only isolations to occur in the samples from the lower levels with any regularity. Of the regularly occurring species some were occasionally isolated at depth but most isolations were from the topsoil fraction. There was also quite

a large number of isolates at 5cm depth. The majority of the species occurred in the top three layers and most of these are to be found in the topsoil and at 5cm depth. At 25cm depth there were only twenty-four species isolated but they occurred in small numbers and generally only once.

The majority also occurred at higher levels. There seems to be only limited toleration of depth and no distinct flora associated with depth as sometimes occurs in other soil types. This may be a feature from the general inhospitality of the spoil material so enhancing the harmful effects of depth.

2.5 Seasonal Variation and Profile Studies from Site 2

2.5.1. Description

Site 2 is located in the centre of the same spoil heap as site 1 which has been described in the previous chapter. The spoil in the centre at site 2 consists only of fine material, there is none of the coarse fraction which was found at site 1. The material on the surface is much darker grey in colour and is very crumbly in texture when dry, but becomes more clay-like when wet. The lower layers are like the same layers in site 1 in texture and colour. It is in the central area of the spoil heap where a few clumps of grass grow (Plates 1a and 1b).

2.5.2. Results and Discussion

The results of sampling are presented in the Appendix Tables 24-35. The general pattern of the fungal population is presented in Fig. 2.10 which shows the numbers of isolates/gram and species isolated at this site. There seems to be some indication of seasonal variation in both isolates and species although it is much more marked in the numbers of

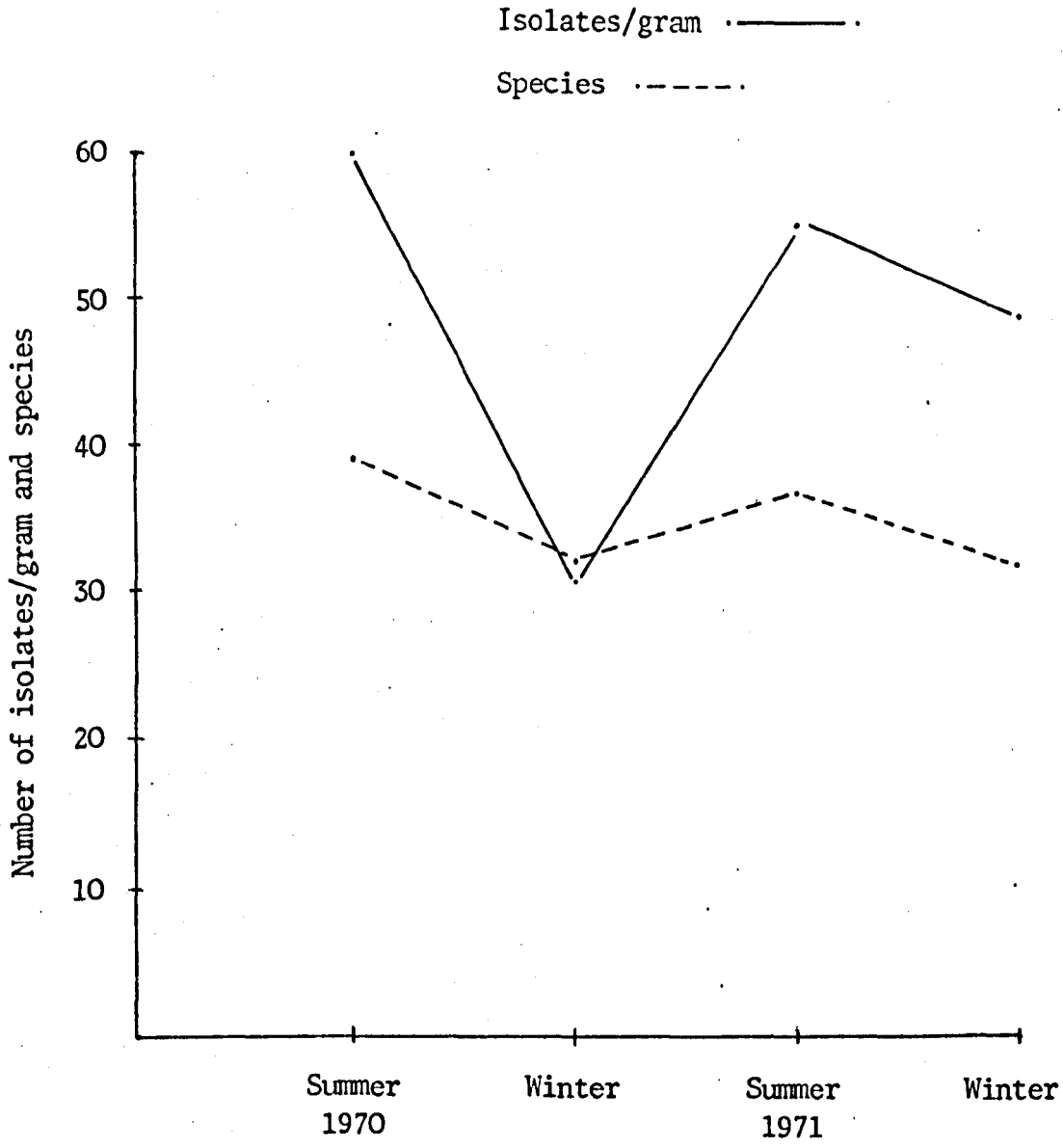


Figure 2.10

Numbers of isolates/gram and species isolated from Site 2 during two years

isolates. It would seem that there is an increase in activity in summer. The very low numbers of isolates in the first winter may have been enhanced by the incubator failure affecting the March sample. However the following winter also contains fewer isolates than the summer samples. The numbers/gram are again much lower than are found in normal soils. The numbers of species follow a roughly similar pattern to the numbers of isolates. Thus in the summer months there is a slight qualitative as well as a quantitative increase.

The fact that the fluctuations of numbers during the two years are similar in the species and isolates is confirmed by the regression line analysis (Fig. 2.11). The correlation coefficient of 0.97 indicates a significance at the 0.1% level which is very high. This suggests that there is little variation in the amount of growth occurring in a species and that any alteration is both qualitative and quantitative.

Graphical analyses of the effect of season and depth on the mycoflora are presented in Figs. 2.12 and 2.13. The numbers of species (Fig. 2.12) show a marked difference between the topsoil and the lower three layers. The lower three levels show very little variation with depth or season possibly as a result of the protection afforded by the covering of the topsoil layer. It may also be that conditions are so harsh that only a few fungi can survive. However, the topsoil fraction does fluctuate a little producing summer peaks of activity and is responsible mainly for the pattern which occurs in the total numbers indicating its relative importance. The numbers of species occurring in the topsoil indicate that it is comparatively favourable for fungal growth. It may be enhanced by the occurrence of airborne contaminants but also is probably being colonised by fungi which are only slowly penetrating the lower levels.

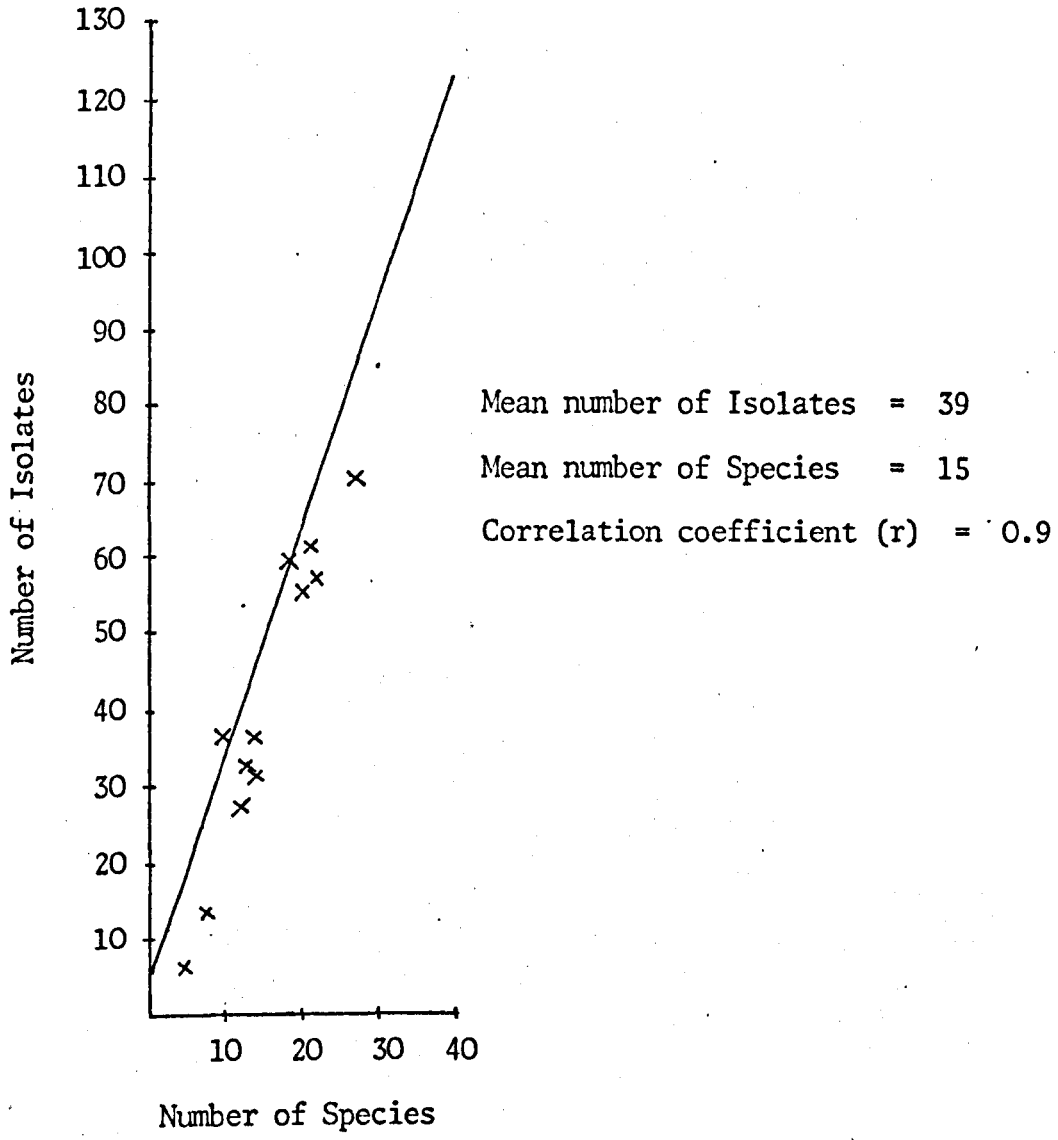


Figure 2.11

Regression line of the number of isolates on the number of species from Site 2

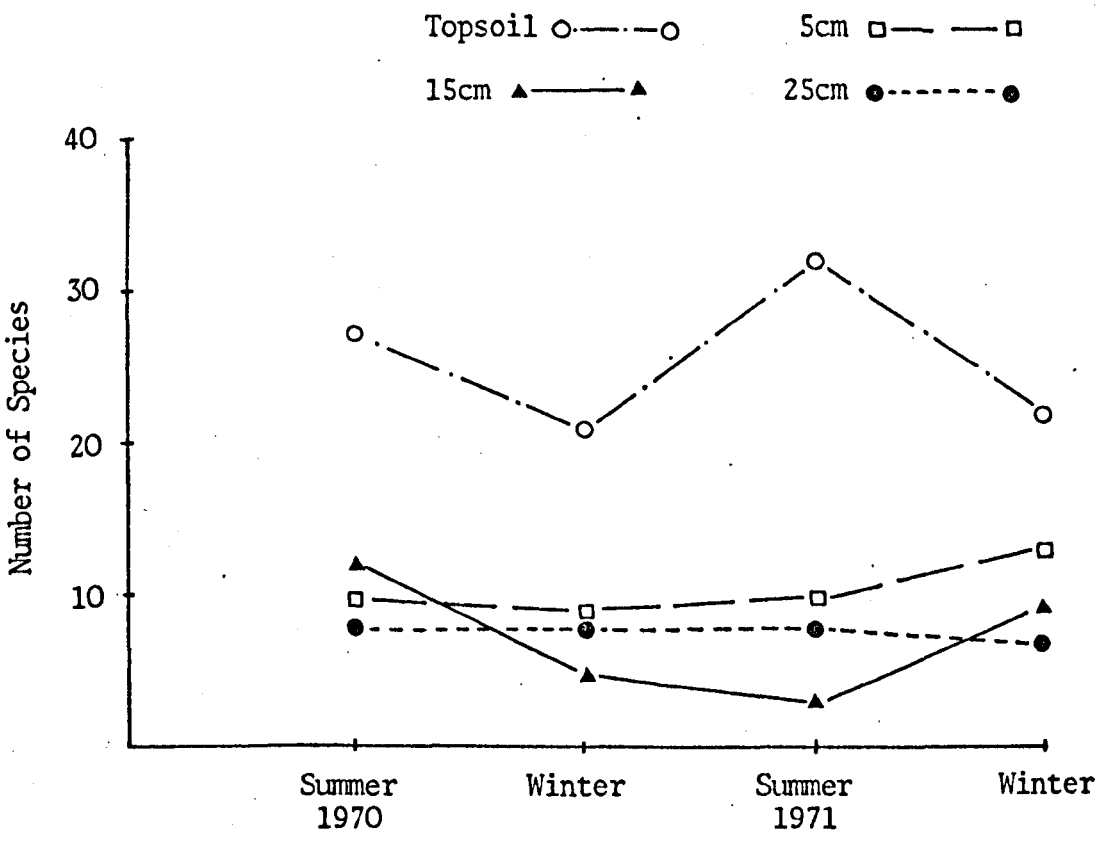


Figure 2.12

Number of species at different depths from Site 2

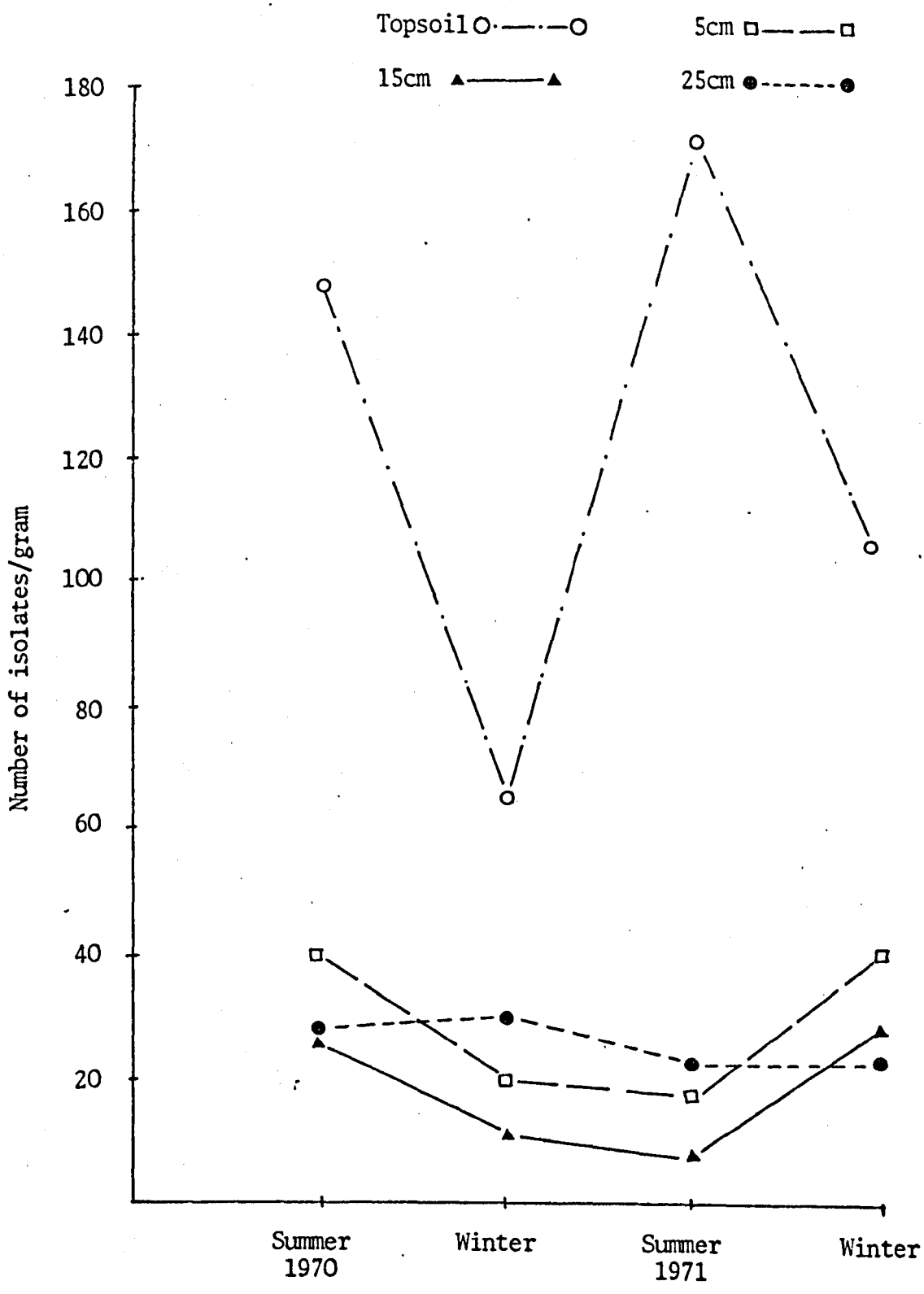


Figure 2.13

Number of isolates/gram at different depths from Site 2

A similar pattern occurs in the isolates/gram found at different depths (Fig. 2.13). There is more variation at 5 and 15cm depths showing no seasonal trends. There was probably some alteration in the conditions at these depths resulting in a decline in the numbers of isolates in the second and third seasons which do not occur in the fourth season which contains similar numbers to the first season. At 25cm depth there is very little fluctuation indicating perhaps the constancy of the conditions at that depth. However there is much fluctuation in the topsoil with marked summer increases. This suggests that the topsoil is affected by seasonal changes, such as temperature and possibly drying. As with the numbers of species the importance of the topsoil is shown by the fact that the pattern is the same as the total numbers of isolates/gram. This illustrates the conclusion that the topsoil is generally a more favourable environment since fungi normally decrease with increasing depth due to alteration in organic content, air and moisture content.

The environmental factors which were measured are presented in the Appendix Table 36 and summarised in Table 2.3. The pH over the two years is generally slightly alkaline and this may have reduced the total numbers of fungi since it is believed that fungi are more favoured by acid conditions. However the season with the most acid range is the first winter which contains comparatively few isolates so that other factors are probably also involved, such as the failure of the incubator which lowered the numbers. It may be that alkalinity was a factor in the second winter when the number of isolates was lower. However this may be a reflection of a slight decline in activity due to seasonal factors. There is very little variation of pH with depth so it is difficult to see if it is having any effect on the numbers at depth. It would seem likely that it is one of the factors influencing the mycoflora but not an overriding one.

Table 2.3

Average lead content, soil moisture and range of pH of soil samples from Site 2

Samples	Lead content (in p.p.m.)		Topsoil		5cm		15cm		25cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
Summer 1970	15,066	17,053	7.0 - 8.4	21.0	7.0 - 8.5	23.0	6.2 - 8.3	22.0	6.8 - 8.3	22.0
Winter	18,333	20,466	6.8 - 7.7	26.0	6.6 - 7.9	28.0	6.6 - 8.1	24.0	6.6 - 8.1	26.0
Summer 1971	14,466	15,733	7.3 - 8.6	26.0	7.2 - 8.5	20.0	7.1 - 8.4	26.0	7.2 - 8.6	21.0
Winter	16,133	22,800	7.7 - 8.2	23.0	8.1 - 8.3	22.0	8.1 - 8.4	24.0	8.1 - 8.4	22.0

Soil moisture which is generally considered to be more directly influential on the soil population does not show any marked seasonal fluctuation or alteration with depth. The topsoil which would be expected to show the most changes holds the water quite well, unlike Site 1 topsoil. This may be due to the texture of the topsoil, which is sandy becoming clayey when it contains water. Another factor is the climate which is fairly constant with regard to rainfall. As there are no extremes in soil moisture it is difficult to determine what effect it may have on this soil population. The difference between the topsoil and the lower levels is much greater in this Site than in Site 1. It also contains more moisture than the topsoil at Site 1. It may be that the moisture held in the upper layers of Site 2, in conjunction with its finer composition, seals off the lower layers from the external environment particularly with regard to gas exchange. This could lead to a more hostile environment with depth and so result in lowering the numbers of fungi which occur there.

The amount of lead extracted with acetic acid from this site did not vary a great deal over the two year period. It was closely associated with the total amount of lead extracted with hydrofluoric/hydrochloric acid, so that the majority of lead may be affecting the biotic sphere. It also indicates that there is little structure in the soil with which the lead can become tightly bound. There is a slight decrease in the amount of lead occurring in the summer samples which may have influenced the increase in numbers which occurs in the summer months. However the differences in lead content and numbers of isolates are relatively small so that it is probably not significant. It would seem that lead also does not exert a direct effect on the numbers during the two years. It

seems a fair conclusion that there is no dominant factor governing the soil mycoflora but rather that they are all interacting to produce the mycoflora. Perhaps the overriding factor is the inhospitality of the environment.

Qualitatively 83 species were isolated during the two year period. However as Table 2.4 shows only a few of these were regular members of the mycoflora and as such can be considered established.

Table 2.4

The number of seasons in which a species occurs

1 season - 52 species
2 seasons - 17 species
3 seasons - 5 species
4 seasons - 9 species

Table 2.4 shows that most (63%) of the species were only isolated in one season and 83% were isolated in two seasons or less. These fungi constitute the occasional flora of this site and as such are probably airborne contaminants. Most of the species belong to the Fungi Imperfecti and may be airborne contaminants or colonisers.

Eight species occurred in all four seasons sampled. They were:- Aspergillus fumigatus (100%), Coniothyrium fuckelii (58%), Epicoccum purpurascens (50%), Penicillium cyclopium (50%), Penicillium frequentans (41%), Penicillium funiculosum (50%), Phoma herbarum (75%), Trichoderma viride (58%), in addition sterile isolates (91%) occurred in all four seasons. The numbers in brackets refer to the percentage of samples in which the fungus occurs. All these fungi are considered to be normal soil inhabitants. Generally they occur in half or more of the individual samples with the exception of P. frequentans. This fungus possibly occurs in low numbers all the year round only occasionally occurring in large

enough numbers to be isolated, following a slight improvement in conditions, Aspergillus fumigatus was the only fungus to be isolated all the time. This would seem to indicate that this fungus is well established occurring in the soil in high numbers regularly. Phoma herbarum was the next most regular member of the mycoflora excluding sterile isolates. The only sample when sterile isolates did not occur was March 1971 which was probably due to the failure of the incubator. Within the main seasons none of these fungi show any regular pattern of seasonal variation occurring at all times of the year. The reason they are not all found in the twelve samples is probably due to the slight fluctuation in numbers due to changes in the micro-environment.

Four of the regularly occurring fungi which contribute more than 5% of the total number of isolates are Aspergillus fumigatus, Coniothyrium fuckelii, Phoma herbarum, Trichoderma viride and in addition there are more than 5% of sterile isolates. This site contains only a limited number of species which are able to overcome the inhospitality of the site in any great numbers.

The effect of depth on the total numbers is very marked, with the majority of isolates coming from the topsoil. At the lower depths the isolates are low in number and irregular in occurrence. At 15cm and 25cm the isolations are generally single and may possibly be due to a stray spore rather than any active population. There are more isolations at 5cm but these are neither numerous nor regular throughout the sampling period. There is no significant flora associated with different depths as there may be in other soils. Considering the species which occur regularly there is generally a decrease in numbers with increasing depth over the two-year period, although at any one sample the numbers may vary at different depths.

2.6 Seasonal Variation and Profile Studies from Site 3

2.6.1. Description

This site is part of an old spoil heap consisting of the 'Tailings' from the lead mine. 'Tailings' are defined as "the finely ground waste from a modern ore-processing plant" (Ford and Rieuwarts 1968). At some time this area has had topsoil added which now remains to a depth of one inch. It is slightly darker in colour than the lower layers (which are a dark sandy colour), and is similar in texture. The soil is a friable sand/clay mixture with sand predominating. It is quite well drained throughout. Acer pseudoplanatus and some Alnus species have been planted here to improve its appearance and are now growing very weakly (Plate 2). If there was any grass planted at the time of the addition of topsoil most of it has now failed. There may havebeen some colonisation from the nearby waste ground giving rise to scattered clumps of grass, however, these could be the remains of the growth from the topsoil. The grass is mainly Festuca rubra which is also not growing very well.

2.6.2. Results and Discussion

The results of the individual samples are presented in Tables 37-48 in the Appendix. Figure 2.14 shows the pattern of the fungal population during the sampling period. It shows that there is no seasonal variation although there is a marked increase in numbers in the second year of sampling. The winter of 1970-71 may have contained more isolates but in one sample the incubator failed and this probably lowered the numbers. This suggests that possibly the summer of 1970 was a poor season containing few isolates. The numbers

PLATE 2 General view of Site 3 with sampling area in the foreground. (July 1971)

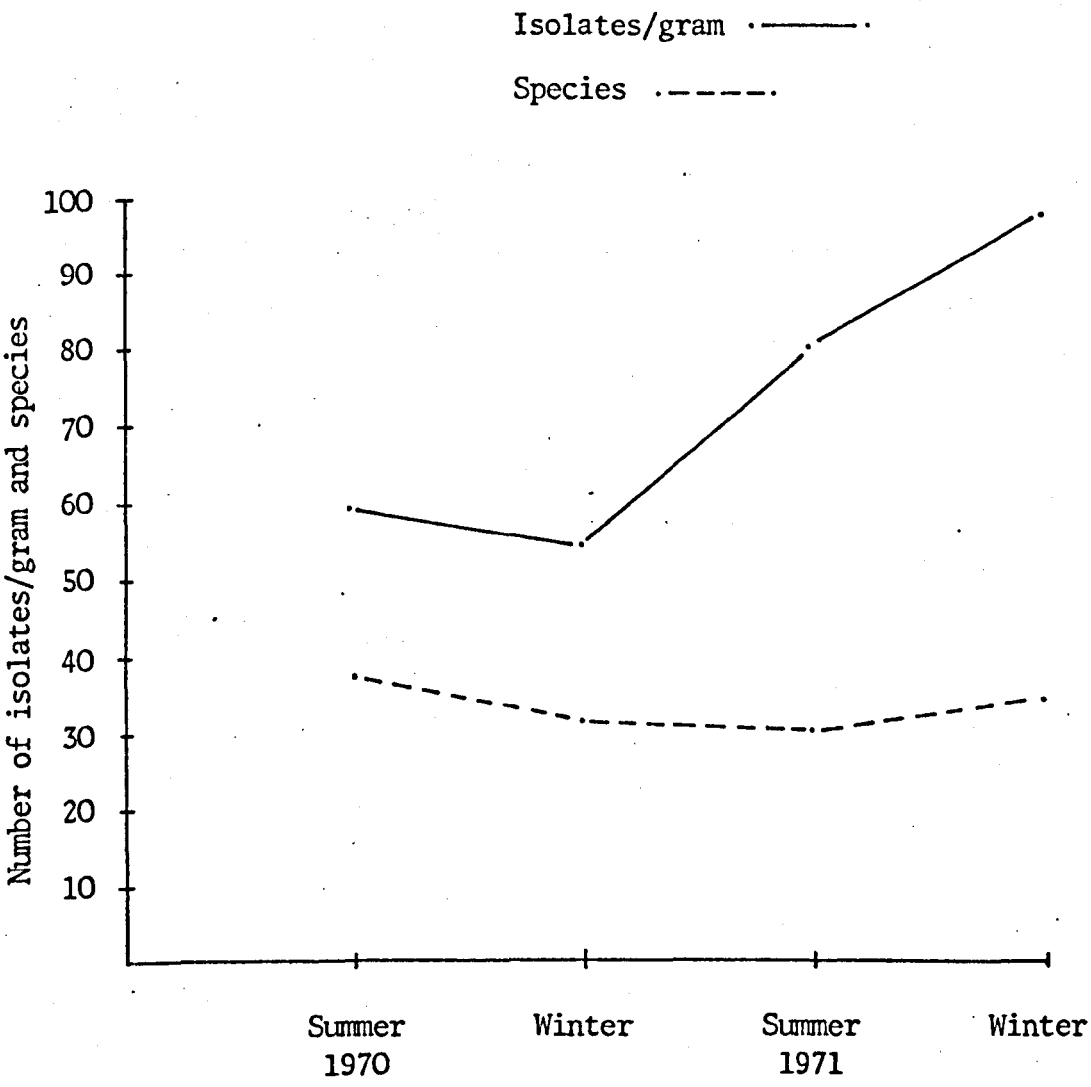


Figure 2.14

Number of isolates/gram and species isolated from Site 3 during two years

of species isolated varies very little over the two years indicating that the environment is fairly stable qualitatively and that the variation is only in the amount of growth allowed. This is confirmed with the regression line which shows that the relationship of the isolates and species is significant at the 5% level only (Fig. 2.15). The relationship at the previous two sites was much closer and although there is only a little more growth here, it is not accompanied by an increase in the numbers of species which was the case in the previous sites. It would seem that the changes in the environment here make it more favourable for these species which are able to exist here but not favourable enough to allow more species to grow.

Graphical analyses of the effect of season and depth on the mycoflora wre presented in Figures 2.16 and 2.17. There is little variation in the numbers of species at different depths. The top-soil fraction generally contains most speceis and shows little variation in numbers during the two years. The lower levels fluctuate more and there is no distinct decrease in numbers with increasing depth. This would seem to suggest that the various levels are subjected to differing influences since there is no pattern. It indicates that depth while influencing the mycoflora to some extent is not an overriding influence.

The numbers of isolates/gram at the different depths (Fig. 2.17) show a similar pattern to the number of species but much more marked. The topsoil fraction contains many more isolates than the lower levels. This would seem to indicate that it is a more favourable environment and that the effect of depth is immediately

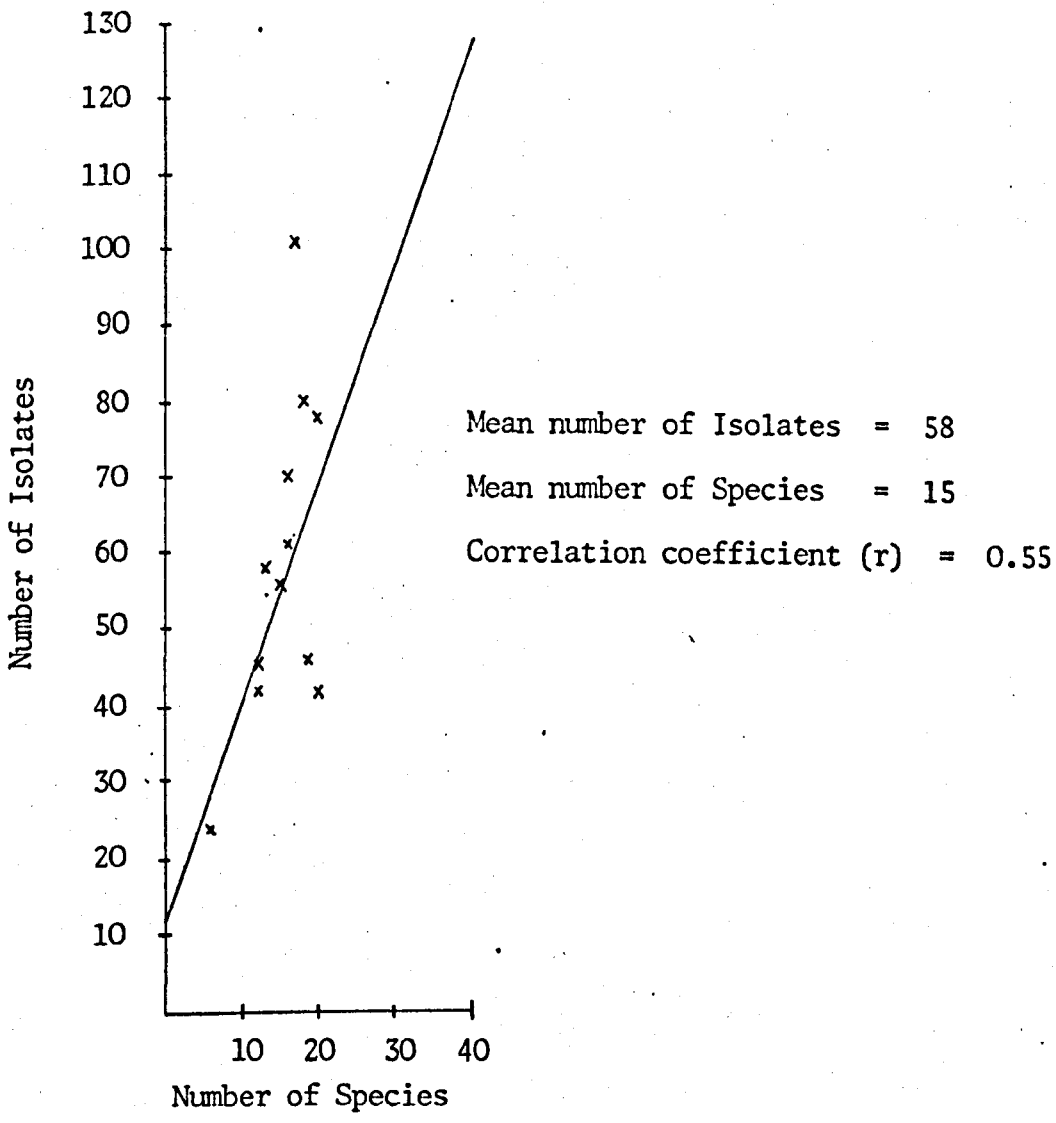


Figure 2.15

Regression line of the number of isolates on the number of species from Site 3

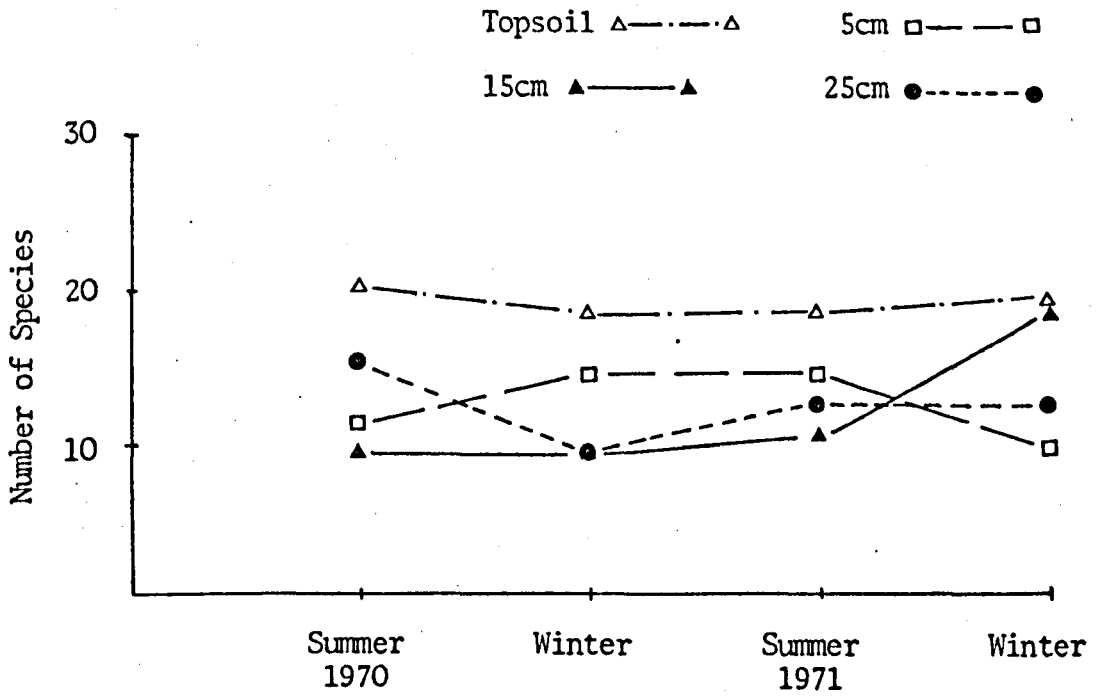


Figure 2.16

Number of species at different depths from Site 3.

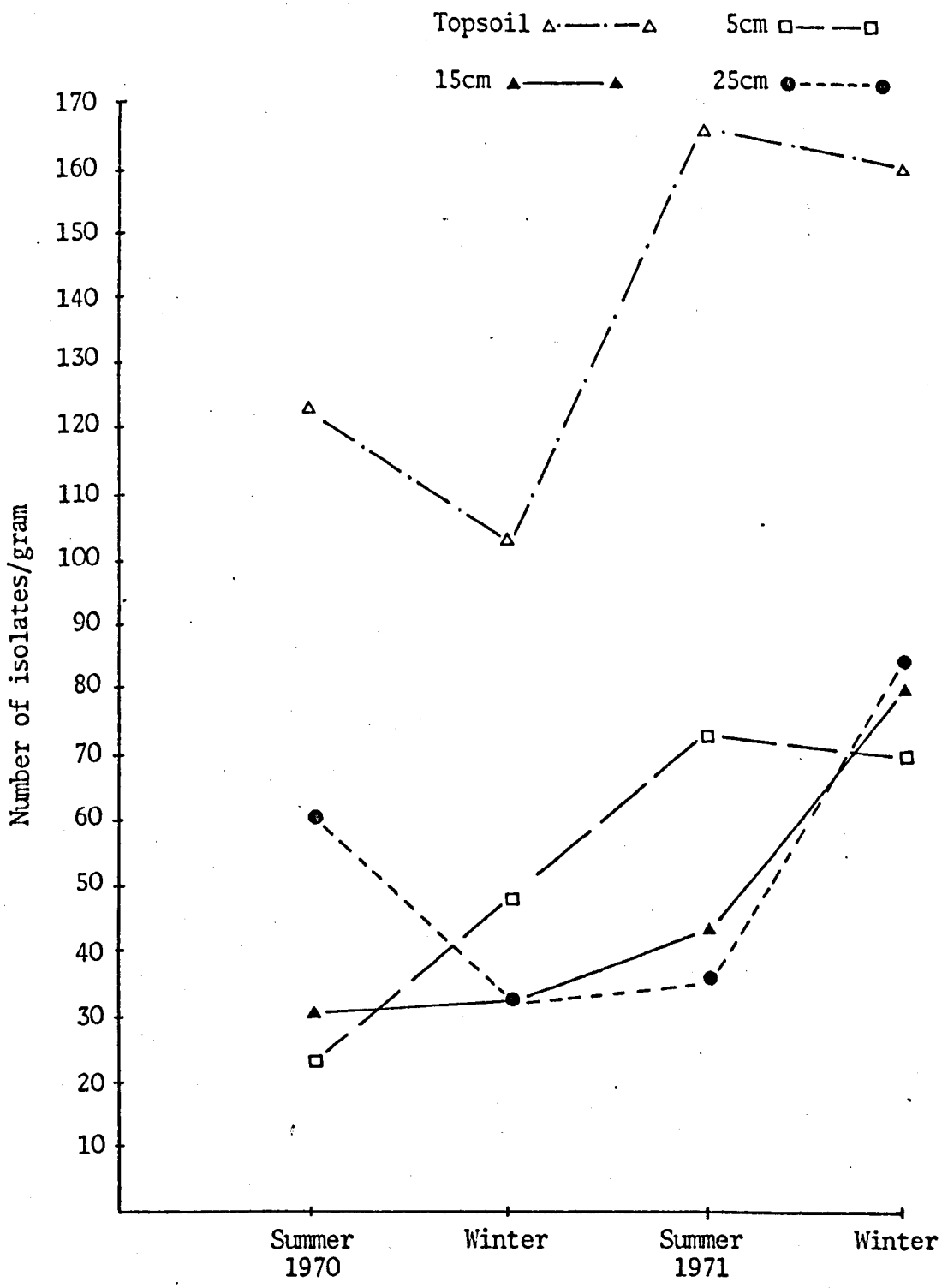


Figure 2.17

Number of isolates/gram at different depths from Site 3

seen below this fraction. At the lower levels the numbers are similar but there are marked fluctuations at the different depths. It suggests that the same factors are not affecting all the levels to give a similar pattern or else the same factors are producing different results. There is some similarity between 15cm and 25cm which may indicate similar conditions occurring in these levels. Apart from the obviously high numbers in the topsoil there is no regular decrease in numbers with increasing depth.

The environmental factors which were measured at each sampling are presented in Table 49 in the Appendix and are summarised in Table 2.5. The pH is generally slightly alkaline although in the first two seasons the lower figure is acidic. This acidity does not seem to have had any effect in increasing numbers of isolates. Possibly the extreme alkalinity of the summer season may have decreased numbers. Later the range of pH is not so extreme remaining slightly alkaline. This coincides with an increase in numbers but the importance of this is difficult to assess. It may be due to interaction of several factors. The pH shows little variation with depth and as such unlikely to be the cause of the differences occurring.

Generally there is little variation in the soil moisture content with depth or season. The spoil holds a fairly constant amount of water and consequently it is difficult to examine the effect of this factor as there are no extreme to compare. There seems to be no direct relationship between the numbers of isolates and the very slight differences in soil moisture. This seems to suggest that conditions of soil moisture are not extreme enough to affect the mycoflora directly.

Table 2.5

Average lead content, soil moisture and range of pH of soil samples from Site 3

Samples	Lead content (in p.p.m.)		Topsoil		5cm		15cm		25cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	H_2O	pH	% H_2O
Summer 1970	24,000	39,300	6.6 - 8.6	11.2	6.4 - 8.5	11.6	6.8 - 8.5	13.6	6.3 - 8.5	9.7
Winter	18,533	41,200	6.8 - 7.6	16.3	6.8 - 7.5	18.0	6.8 - 7.3	20.0	6.8 - 7.3	15.3
Summer 1971	21,133	39.866	7.2 - 7.6	13.6	7.3 - 7.4	13.5	7.3 - 7.5	12.0	7.3 - 7.5	17.3
Winter	22,533	30,000	7.1 - 7.7	14.8	7.2 - 7.8	13.0	7.1 - 7.9	14.8	7.4 - 7.8	14.3

This spoil heap has a relatively high lead content, the Hac extractable lead is much less than the total lead content. The lead content does not vary very much over the two years with the exception of the first winter which contained less than the other samples. There is no marked increase in numbers as would be expected, however the incubator failed in the March 1971 sample lowering the numbers and this may have masked any effect of the lead content. It is impossible to say therefore whether the lead may act differentially on the numbers as well as generally lowering the numbers isolated.

During the two year sampling period 84 different species were isolated, most of them belong to the Fungi Imperfecti with the Penicillia contributing a large proportion. The Penicillia occur regularly throughout the sampling period with slight increases in the winter months due to growth of existing species rather than an increase in species. There are several species of Phycomycetes isolated during the sampling period, these tending to occur mainly in the winter months. Most of the species isolated occur in one season and only a very few occur with regularity.

Table 2.6.

The number of seasons in which a species occurs

1 season	-	55 species
2 seasons	-	16 species
3 seasons	-	8 species
4 seasons	-	5 species

The five species which occur regularly are:- Aspergillus fumigatus (100%) Coniothyrium fuckelii (66%), Penicillium cyclopium (58%), Phoma herbarum (83%) and sterile isolates (100%). The numbers in brackets refer to the numbers of individual samples in which the species occurs. Two other species occur in six samples but only three seasons and they are Penicillium fumiculosum and yeast species. All these fungi are considered to be normal soil inhabitants. Sterile isolates are very regular in numbers and occurrence, and generally constitute a large part of soil floras. Aspergillus fumigatus occurs in every sample with fairly constant numbers. This suggests that this fungus is well established at this site, although it is of course favoured by the use of soil plates. Coniothyrium fuckelii, Penicillium cyclopium and P. funiculosum all show a tendency to occur in the winter months of sampling, however they do not occur in very large numbers of isolates. These fungi can obviously survive in this soil at all times but the numbers fluctuate so that they are not isolatable on all occasions. Yeast species occur in low numbers with no seasonal variation. Phoma herbarum occurs in widely varying numbers and constitutes quite a large fraction of the total isolations. The pattern of isolation for this fungus is similar to the total shown in Figure 2.14 but decreases slightly in the second winter.

There are only two species which contribute more than 5% of the total, viz. Aspergillus fumigatus and Phoma herbarum. In addition to these the sterile isolates also contribute a large percentage to the total. These three are so dominant that they contribute nearly 40% of the total isolations from the two years

of sampling. It would seem that, while these fungi are well established, there is not yet a settled flora for this site, unless it is so inhospitable that only these species can exist there. A. fumigatus and P. herbarum are better able to adapt to or tolerate these conditions. The lack of competition may also help them to occur in such relative profusion. The majority of the isolations occur only occasionally and do not individually contribute many isolates even when they occur regularly.

Depth exerts a marked effect on the total numbers of isolates and species for the two-year period with the topsoil containing many more than the lower levels, all of which are very similar in numbers. Of each species there are generally few isolates and most show no marked influence of depth. At these levels there does not seem to be a particular flora associated with depth. Some species show a preference for the topsoil, although they may occur at the lower levels - Mucor hiemalis, Fusarium sambucinum var. caeruleum, Phoma glomerata. Several of the regularly occurring species are found at all levels with no marked preference. However, Coniothyrium fuckelii occurs mainly in the topsoil although there are quite a few isolates from 25 cm depth. Penicillium simplicissimum is only found at 25cm depth. Phoma herbarum occurs in greater numbers in the topsoil than the other depth whereas Aspergillus fumigatus is found in very similar numbers at all levels. In most cases the numbers are so small that the isolation at a certain depth could be due to chance rather than a regular occurrence.

2.7 Seasonal Variation and Profile Studies at Site 4

2.7.1. Description

This is an area of rough mine waste which has been levelled. The area is enclosed by a large spoil heap on one side and brick walls on the other sides (Plate 3a). The soil is coarse and pebbly becoming more so with depth. Also, there is a very hard layer at approximately 9ins depth so that no samples could be taken below this level. There have been attempts to make the area more attractive and several trees (Fraxinus excelsior, Acer pseudoplanatus, Malus sp., Aesculus hippocastanum, Salix sp. and Tilia sp.) have been planted which are growing relatively well (Plate 3a). In December 1970 topsoil was added to a depth of approximately 2ins. Prior to this the ground cover had been very limited with only a few clumps of grass surviving. After the addition of topsoil, grass grew fairly well giving continuous cover, which had to be mown in the summer months (Plate 3b). The plants now found in addition to the trees were:- Festuca rubra, Anthoxanthum odoratum, Rumex sp., Stellaria media, Ranunculus bulbosus and Trifolium repens. There was some growth of mosses amongst the ground cover.

2.7.2. Results and Discussion

The results of individual samples are presented in Tables 50-61 in the Appendix. The results for this site have been complicated by the addition of topsoil in December 1970. This meant that there was only a limited period before the application, for comparison with samples taken afterwards. The addition of topsoil has several possible effects. It may introduce a new supply of nutrients and also additional inoculum which may contain some different fungi. There

(a) General view of Site 4 (July 1971)

PLATE 3

(b) Close up view of Site 4 (July 1971)

is also the physical aspect of covering the previous layer of topsoil and causing an alteration in the depth of the other levels. In this case the original topsoil was sampled at approximately 8cm and the 5cm at 15cm. The covering layer also means that the lower layers will be protected from environmental factors such as temperature, evaporation, wind. This means that the results for the first winter include one sample at 15cm depth and two of the added topsoil. For the numbers of isolates/gram the results for the 15cm sample have been omitted. Instead an average of the two results from the Added topsoil was taken and used so that the sample is similar to those following. However, this cannot be done for the numbers of species so that they consist of the actual sample results and this must be borne in mind when considering the results.

One of the effects of the addition of the topsoil is shown in both Figures 2.18 and 2.19 where the numbers of isolates and species are higher after the addition of the topsoil. There was an immediate increase in the Winter of 1970-71 and this was continued in the Summer of 1971 when the soil had settled and the surface vegetation was becoming established. The summer increase may be due to seasonal factors since in the following winter numbers fall to those of the previous winter. This could be a seasonal change related to the surface vegetation. Alternatively the numbers may be settling to an established pattern as the new site is effective. That is that the lower layers which consist of lead contaminated spoil which is poor may be mixing with the added topsoil and also there may be loss of nutrients downwards through the soil. Despite the addition of topsoil the numbers of isolates/gram are much lower than are generally considered to occur in normal soils. Warcup (1951) in his study of five soils found that the numbers ranged from 36,000 to 425,000 colonies/gram.

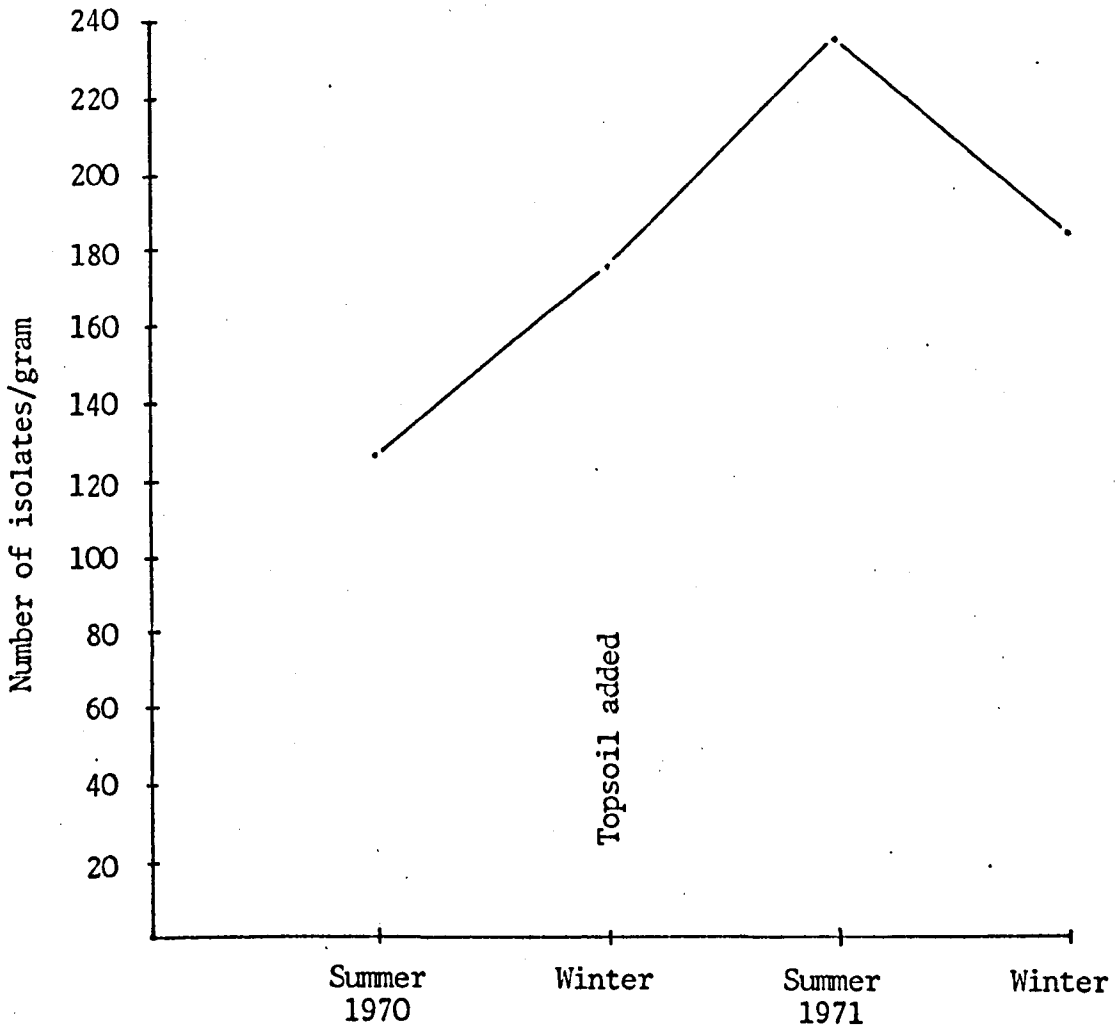


Figure 2.18

Number of isolates/gram isolated from Site 4 during two years

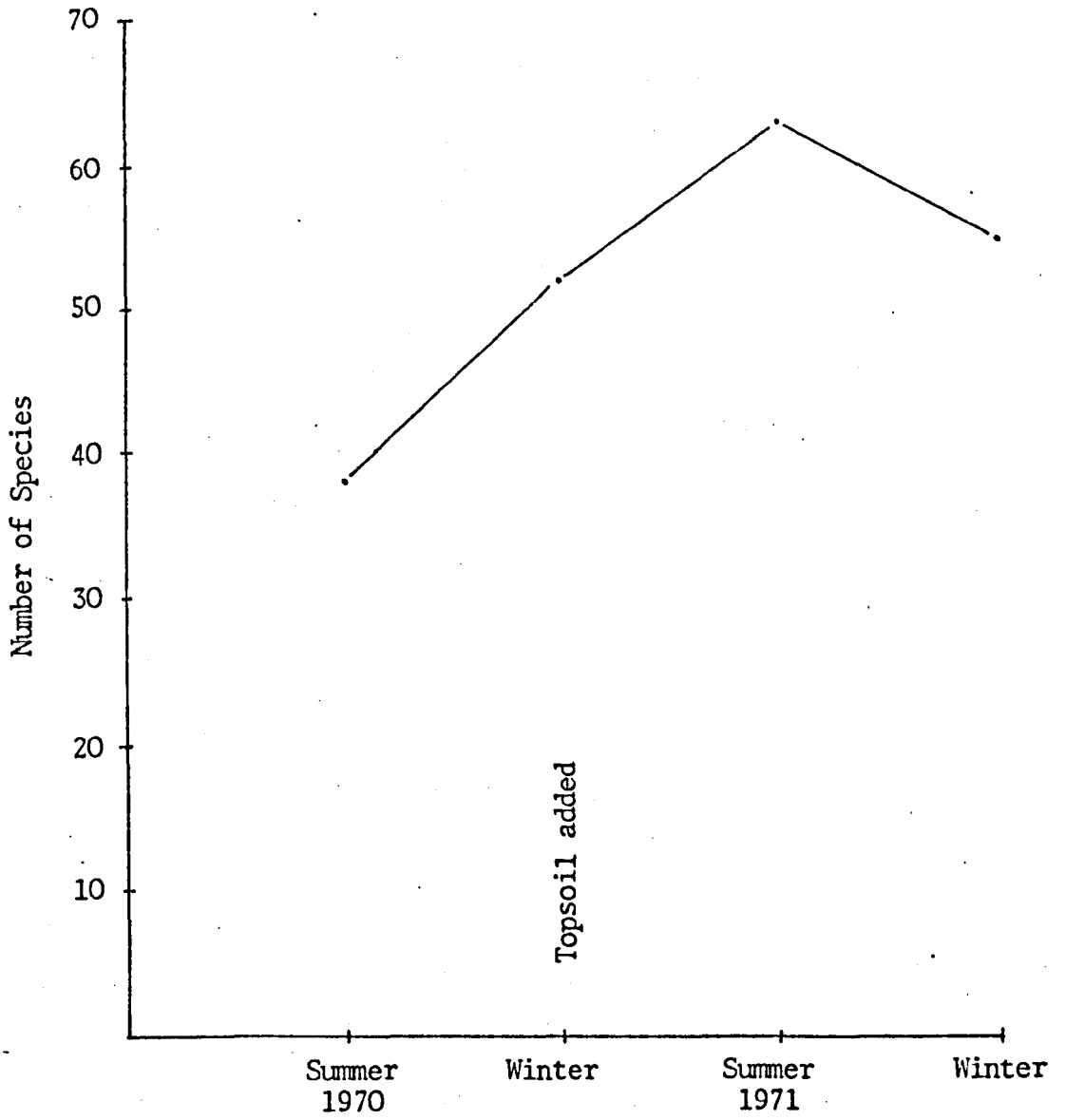


Figure 2.19

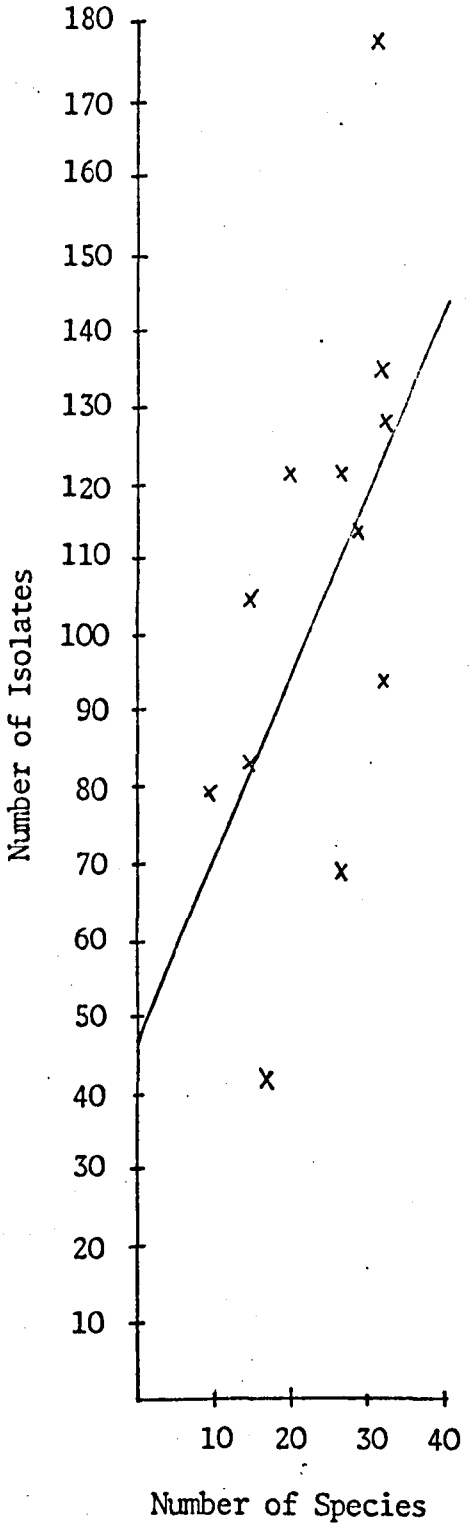
Number of species isolated from Site 4 during two years

The numbers of species (Fig. 2.19) follow a similar pattern to the numbers of isolates and are probably subject to the same influences. Some of the species which grew initially after addition may in fact be unable to exist in this environment. However, the addition of topsoil has added new species either from inoculum or by providing an environment in which new species could grow.

Although there seems to be a similar pattern in the numbers of isolates and species the regression line analysis shows that the relationship is significant only at the 5% level (Fig. 2.20). So that although the numbers are similar in general there is still much variation in the numbers of isolates/species. This indicates that there is a varied amount of growth going on, possibly with some species hardly growing at all while others are able to flourish.

Graphical analyses of the effect of season and depth on the numbers of species and isolates are presented in Figures 2.21 and 2.22. It is generally considered that increasing depth is associated with decreasing numbers of isolates and species as a result of increased carbon dioxide, less oxygen and insufficient organic matter. However with regard to the numbers of species there is little evidence of a regular decrease. At 5cm depth the numbers are relatively stable which possibly reflects the state of the conditions at this level. The former topsoil may have been influenced by the addition of the new topsoil since in the seasons following the numbers were higher than in Summer 1970. The added topsoil also has a similar pattern to the total which is indicative of its contribution. The numbers which occur in Winter 1970-71 are probably lower than they would have been if it had been possible to take three samples. However, in only two samples it contains a relatively large number of species.

The numbers of isolates (Fig. 2.22) show a distinct decrease with



Mean number of Isolates = 105

Mean number of Species = 24

Correlation coefficient (r) = 0.56

Figure 2.20

Regression line of the number of isolates on the number of species from Site 4

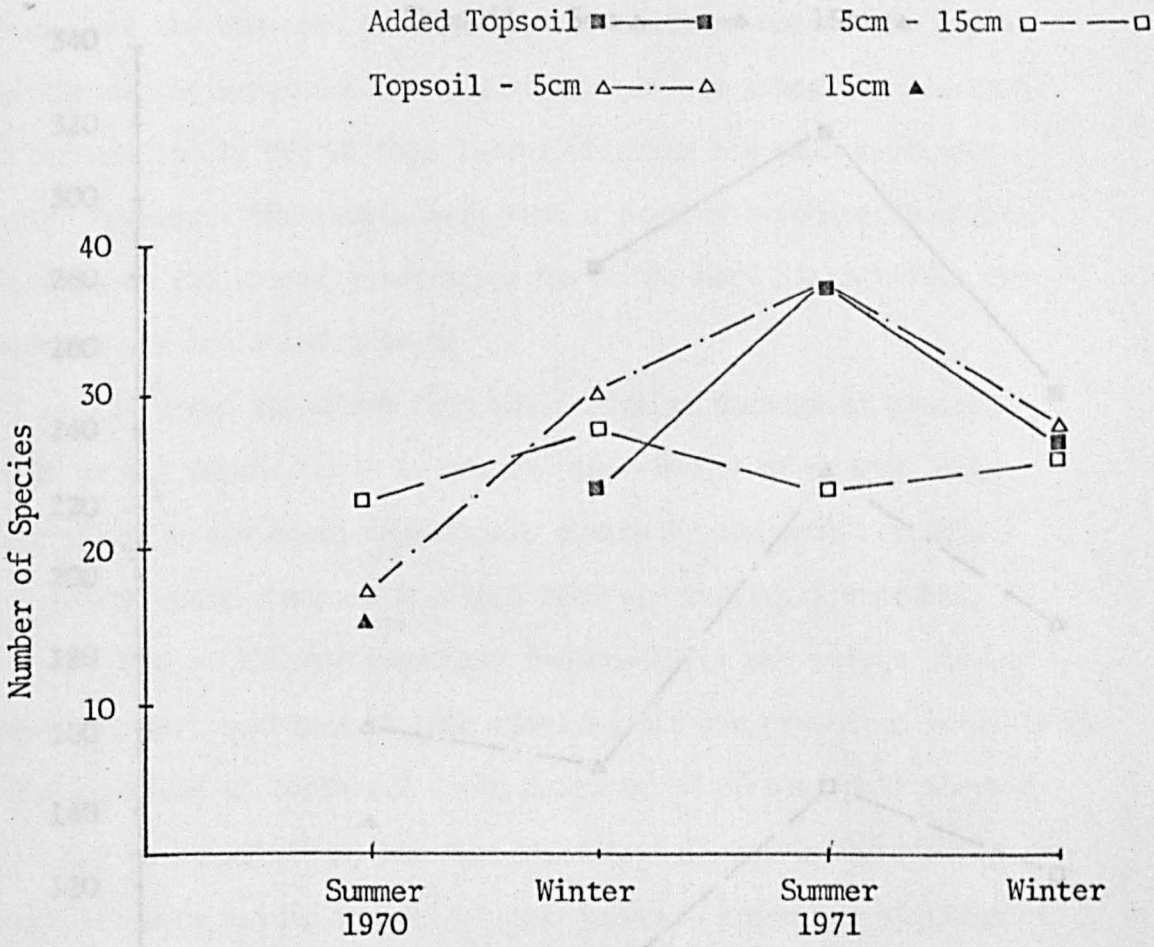


Figure 2.21

Number of species from different depths from Site 4

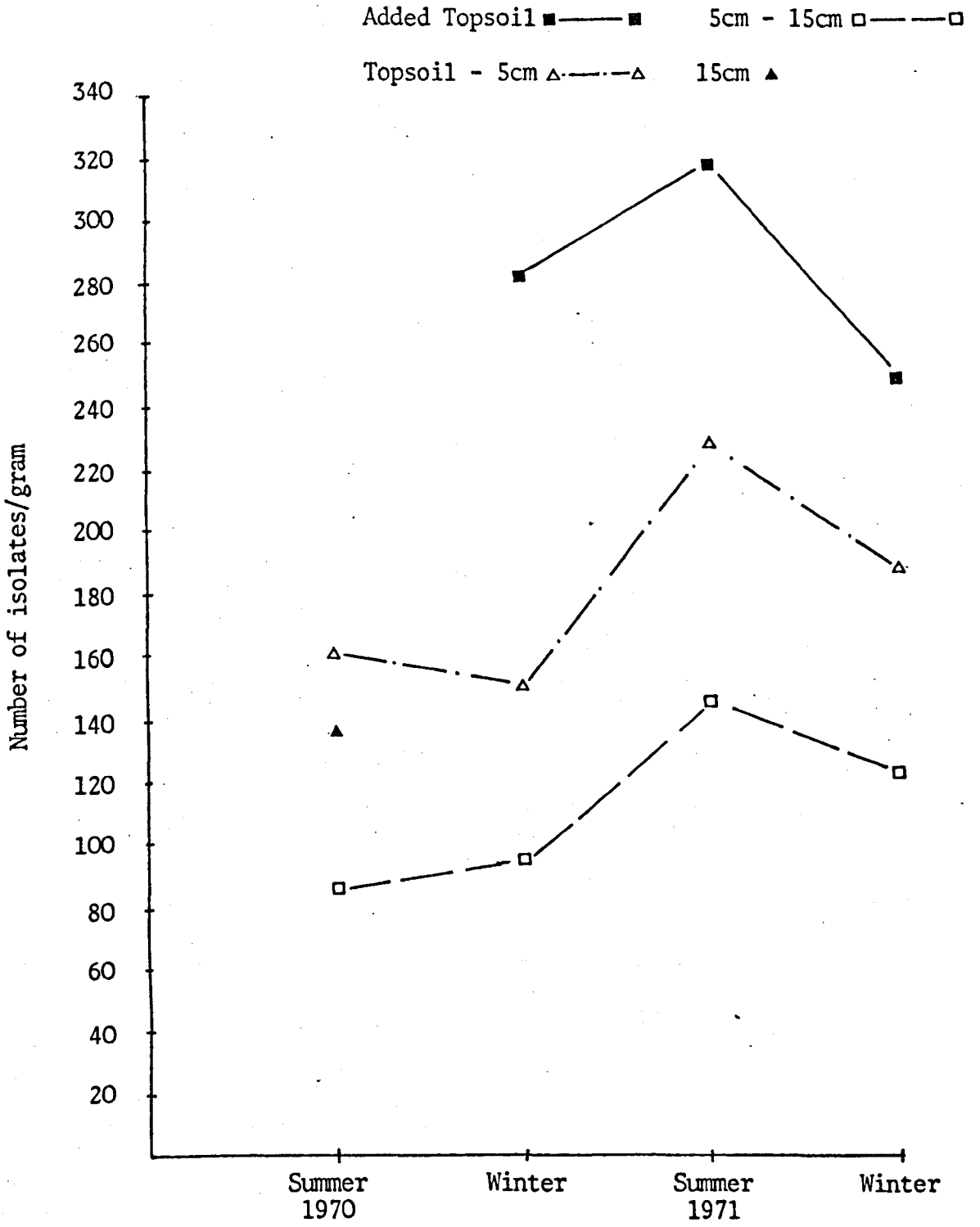


Figure 2.22

Number of isolates/gram at different depths from Site 4

increasing depth. However all the horizons show a similar pattern of isolates during the two years. This suggests that similar influences are active at each level. Although the response is reduced at the 5cm then 15cm level, which may be due to the depth. All the depths sampled contained higher numbers after the addition of topsoil and it may be this factor which is the main influence on the numbers. This would mean that a smaller response would be expected at the lowest level since it is the most distant from the influence of the added topsoil.

It seems therefore that while similar numbers of species exist at all depths there is not the same amount of growth. The conditions in the added topsoil are obviously the most suitable for growth while those at 5cm then 15cm are the least suitable.

Some of the environmental factors which may affect the mycoflora were measured at each sampling and are presented in Table 62 and summarised in Table 2.7. The range of pH of the spoil shows a tendency to be alkaline, but the added topsoil has a wider range generally more acidic than the other layers. After the addition of topsoil the lower layers become definitely alkaline. The total numbers of isolates and species show no reaction which could be associated directly with pH, although the general alkalinity may have reduced the numbers in the samples. With regard to the individual horizons there seems to be little or no direct relationship between the pH and the numbers of isolates and species. It may be that the stimulation from the added topsoil has masked any direct influence of pH.

The soil moisture content does not vary a great deal with the changing season. There is some variation with depth, generally the amount of moisture decreases. At first the topsoil contained

Table 2.7

Average lead content, soil moisture and range of pH of soil samples from Site 4

Samples	Lead content (in p.p.m.)		Added Topsoil		Topsoil - 5cm		5cm - 15cm		15cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
Summer 1970	10,333	28,800			6.3 - 8.4	10	7.5 - 8.5	7	7.1 - 8.5	7
Winter	1,333	12,800	5.2 - 7.1	27*	6.4 - 7.6	19	6.6 - 7.9	6		
Summer 1971	2,566	9,166	6.6 - 8.1	16	7.2 - 7.9	7	7.2 - 7.5	5		
Winter	2,250	9,300	6.8 - 7.4	13	7.2 - 7.9	7	7.0 - 8.0	5		

* average of two samples

more moisture than the lower levels and then after the addition of topsoil the amount was similar at the 5cm then 15cm and the topsoil then 5cm levels. The amount in the added topsoil gradually decreased during the sampling period. These fluctuations are probably indicative of the soil settling into a new regime imposed by the added topsoil since the rainfall of this area is fairly constant throughout the year, although the moisture does depend on the conditions immediately prior to sampling. There is a varied reaction to the soil moisture, which is probably complicated by the addition of topsoil and other factors. The added topsoil shows no direct relationship between moisture and numbers of isolates. At 5cm depth no relationship can be discerned. In the topsoil fraction there is an indication that reduced moisture content allows more growth. This can only be suggestive since it coincides with the addition of topsoil which is also probably involved in increasing the numbers. It would seem that soil moisture is involved in influencing the mycoflora but not exclusively.

The added topsoil was shown to be comparatively lead free when it was analysed in November 1971, however, there had probably been some mixing of the layers so imparting some lead to the added soil. After the addition of topsoil the total figure for the amount of lead, particularly the Hac extractable lead, was lowered. This figure is a composite figure from a mixture of all the layers sampled. There is quite a difference in the total lead content and the Hac extractable lead which may reflect the structure of the soil at this site. Since it would seem that there is some structure which can bind the lead quite tightly so removing some of it from circulation. The lead content remains fairly constant after the initial drop in the first winter. The numbers of fungi vary over this period indicating that other factors are involved. However, the numbers do increase in these

samples with a lower lead content but this may also have been a response to the addition of topsoil. It may have been this addition of 'clean' soil providing a 'healthy' environment containing large numbers of isolates and exerting a beneficial effect on the lower layers which was the major factor, a part of which was the reduced lead content. The lead content is probably exerting a general effect on the mycoflora lowering the numbers in comparison with other soils rather than having any specific effect.

During the two-year period of investigation 118 different species were isolated, most of them belonging to the Fungi Imperfecti with quite a high proportion of Penicillia. However, many of the species were not regular members of the mycoflora occurring in small numbers and only in occasional samples. There are only a few species which were isolated regularly as shown by Table 2.8.

Table 2.8

Number of seasons in which a species occurs

1 season - 59 species
2 seasons - 35 species
3 seasons - 14 species
4 seasons - 10 species

It is likely that most of the species occurring in three seasons and possibly some in two are in fact present in the soil at other times, but not in quantities which allow their isolation on the soil plate. It may also be that some, in fact, do die out possibly to become established again.

The ten fungi which occurred in the four seasons sampled were:- Absidia spinosa (58%), Aspergillus fumigatus (66%), Coniothyrium fuckelii (66%), Cylindrocarpon olidum (33%), Gliocladium

roseum (75%), Mucor varians (41%), Penicillium funiculosum (50%), Phoma herbarum (91%), Trichoderma viride (83%) and sterile isolates (100%). The numbers in brackets are the number of individual samples in which a fungus occurred. Three other species occured in six or more individual samples but only in three seasons and they were Fusarium sambucinum var. caeruleum (66%), Mucor hiemalis (50%) and Penicillium lilacinum (50%). All these species are considered to be regular soil inhabitants. Also, all of these fungi occur before and after the addition of topsoil, although their growth may have been affected by the addition of topsoil.

Penicillium lilacinum occurred in relatively large numbers before the addition of topsoil, this may be due to its tolerance of many chemicals (Raper and Thom 1949) and lack of competition would have been altered by the application. Trichoderma viride occurred in greater numbers after the addition of topsoil indicating its predominance in 'clean' soils as compared with lead contaminated soils. Mucor hiemalis, being a simple sugar fungus, also occurred mainly after the addition of topsoil, possibly owing to the influx of nutrients.

Both Coniothyrium fuckelii and Gliocladium roseum showed an increase in numbers in the winter months. Prior to the addition of topsoil they generally occurred in the winter months, afterwards they occurred more regularly but still with a winter increase. This may be due to many other fungi not occurring so frequently in winter so that more isolates of these species can develop on the soil plates, or it may be associated with the decomposition of nutrients supplied with the added topsoil. Phoma herbarum occurred in greater numbers in the winter months mainly before the addition of topsoil, afterwards the peak was not so high. This may have been due to increased

competition in the soil or on the soil plate lowering the numbers for several samples after the addition. Another regularly occurring fungus had an interesting distribution over the two years - Aspergillus fumigatus. This fungus is considered to be a common inhabitant of soils and yet it declined in numbers until there were no isolations immediately after the addition of topsoil. This is unlikely to be due to competition in the soil or on the soil plate as it is considered to be one of the favoured species.

Two fungi which occur in all four seasons do not occur in many individual samples - Cylindrocarpon olidum and Mucor varians. This indicates that these fungi are probably regularly occurring in the soil but only in small numbers. Occasionally the numbers increase sufficiently to be isolated on the soil plates. These fungi are perhaps unable to grow very well as yet but may be able to adapt to the conditions. Alternatively, they may have only a restricted micro-habitat which is not always present in the soil sampled. The remainder of the constant species showed no distribution variations in their isolates.

The fungi which individually constitute 5% or more of the total are:-Coniothyrium fuckelii, Gliocladium roseum, Phoma herbarum, Trichoderma viride and sterile mycelia. These fungi are all normal soil inhabitants and occurred regularly throughout the sampling period. It is interesting that they are two types of fungi, G. roseum and T. viride are both heavily sporing Moniliales which are important in 'clean' soils, while P. herbarum and C. fuckelii are both Sphaeropsidales and usually found in normal soils only in low numbers. This might reflect the two differing soils involved in this site. First there was the spoil material which was then covered with agricultural loam. It may have been the influence of these two

soils which resulted in these species occurring in such large numbers. This is also suggested by their distribution through the soil profile.

In considering the effect of depth direct comparisons must be made with the varied number of samples at different depths. The added topsoil was sampled eight times and the 15cm level four times during the two years. However, despite the variation in samples the added topsoil contains the most numbers of isolates which would be expected as it is relatively lead free and a fairly good soil. The original topsoil and 5cm are very similar in numbers. Most of the regular and frequent species occur at all levels in varying amounts. The numbers may vary in different samples with more isolates occurring at the lower levels, but generally the numbers decrease with increasing depth. Aspergillus fumigatus, Penicillium funiculosum, and Penicillium canescens occur at all levels in similar amounts indicating that depth has little effect on these species. Trichoderma viride shows a marked decrease with depth and is present in very large numbers in the added topsoil. Penicillium luteum and Penicillium simplicissimum also have a marked preference for the added topsoil while Mucor hiemalis and Gliocladium roseum have similar numbers in the added topsoil and the now 5cm layer. So that most of these fungi prefer the added topsoil and show a decrease with depth indicating that the numbers are influenced by the conditions at lower levels. Also, as they show a preference for the added soil these fungi may be less tolerant of the lead in the lower levels.

However, Coniothyrium fuckelii, Penicillium lilacinum and Phoma herbarum seem to exist in the lower levels mainly as that is where the most isolations appear. These fungi are possibly better

equipped to tolerate the lead present at these levels which are also probably poorer in nutrients, oxygen, etc. They may be assisted by the apparent lack of competition from other fungi. P. lilacinum in particular occurs in large numbers at 15cm depth and must have high tolerance.

Although in general terms there is a decrease in numbers with increasing depth, there does not seem to be a particular flora associated with each horizon as most of the isolates are few in number and many species are not isolated frequently. The topsoil and 5cm depths do have many species in common and are very similar in numbers.

The addition of topsoil has affected the site in many ways, physically and biologically. Physically there is the alteration of depth and the protection of the lower levels and all the changes associated, which have been mentioned earlier. Biologically, the topsoil may have introduced added and different inoculum. There is relatively little movement of species from the topsoil to lower levels as there are few species which occur in the first instance in the topsoil sample only. Also the topsoil probably introduced additional nutrients which is probably the reason for the bursts of activity at different depths as the effect is felt, during the summer months. In the development of a rhizosphere from the grass cover there would be added nutrients as the roots move down. An additional feature of the added topsoil was that it supplied a comparatively lead free environment in which the mycoflora could flourish and so act as a possible reservoir of supply to the lower levels. It is difficult to assess the true effect of the addition of the topsoil as it was added before a whole season's samples had been taken so preventing actual direct comparison.

2.8 Seasonal and Profile Studies from Site 5

2.8.1. Description

This sampling area is part of an agricultural field which is near to the site of the mine and smelter and as a result it may have been exposed to dust from the extraction procedures. In addition to the possible aerial contamination it may contain lead as a result of water draining from nearby veins. The field is lower than the other sites and is nearer the River Derwent. The soil is dark, fairly heavy and clayey, holds much water (Plate 4b) and consequently it is usually very clumpy in texture.

The field is ploughed and then left fallow in winter and Barley grown in spring and summer (growing season may extend from March to September) (Plate 4a and b). The Barley is contaminated with lead and can only be fed to stock when mixed with clean corn and as a result, the field had to be bought by the smelting company. The actual sampling area is at the edge of the field between the sown Barley and the grass verge where there is usually some bare soil which was easier to sample than amongst the corn.

2.8.2. Results and Discussion

The results of each sample are presented in Tables 63-74 in the Appendix. The amount of soil from this site used on the soil plates was much less than for the other sites on account of the much larger numbers of fungi isolated from this site in an exploratory study. The amount of soil used in making up the soil plates was 10mg and this must be borne in mind when considering the results. This is particularly so with regard to the qualitative results since the numbers of species cannot be multiplied to a standard figure unlike the numbers of isolates which are presented as isolates/gram.

(a) View of Site 5 - sampling site between crop and hedgerow. (July 1971)

PLATE 4

(b) View of Site 5 in winter (January 1972)

The total numbers of isolates/gram (Fig. 2.23) are much higher than the spoil heap figures discussed in previous sections. However, they are lower than figures for other soils, as in alkaline grasslands Warcup (1951) found the number of colonies were 36,000 at pH 8 and 59,000 in soils with pH 7.5. Over the two years the numbers of isolates/gram show a decline which hinders the identification of any seasonal variation. It may be that the summer of 1970 was a very favourable season encouraging fungal growth and that the winter of 1971/72 was particularly harsh so giving rise to the great disparity in the numbers unlike the intervening seasons. Another factor which may have affected the results slightly in the early samples may be an increased efficiency in excising the colonies from the plates so not allowing the fast growers to spread and give rise to a large number of isolates. The numbers of species (Fig. 2.24) do not show much variation over the two years. There is a slight decrease in the winter months. It would seem that the differences between two summer seasons and the two winter seasons is due to variation in the amount of growth of the species present. Although there is a difference in the numbers of species present at different seasons there is not always a difference in the numbers of isolates. There is a similar number of isolates occurring in Winter 1970/71 and Summer 1971 due, it would seem, to greater growth occurring in the winter months which may indicate that conditions were more favourable at that time than in the following summer months.

The relationship between the numbers of isolates and species has been examined statistically using regression line analysis (Fig. 2.25). There was much variation around the regression line as shown by the graph. There is, however, a significant relationship between the numbers of isolates and species, which is significant at the 2% level.

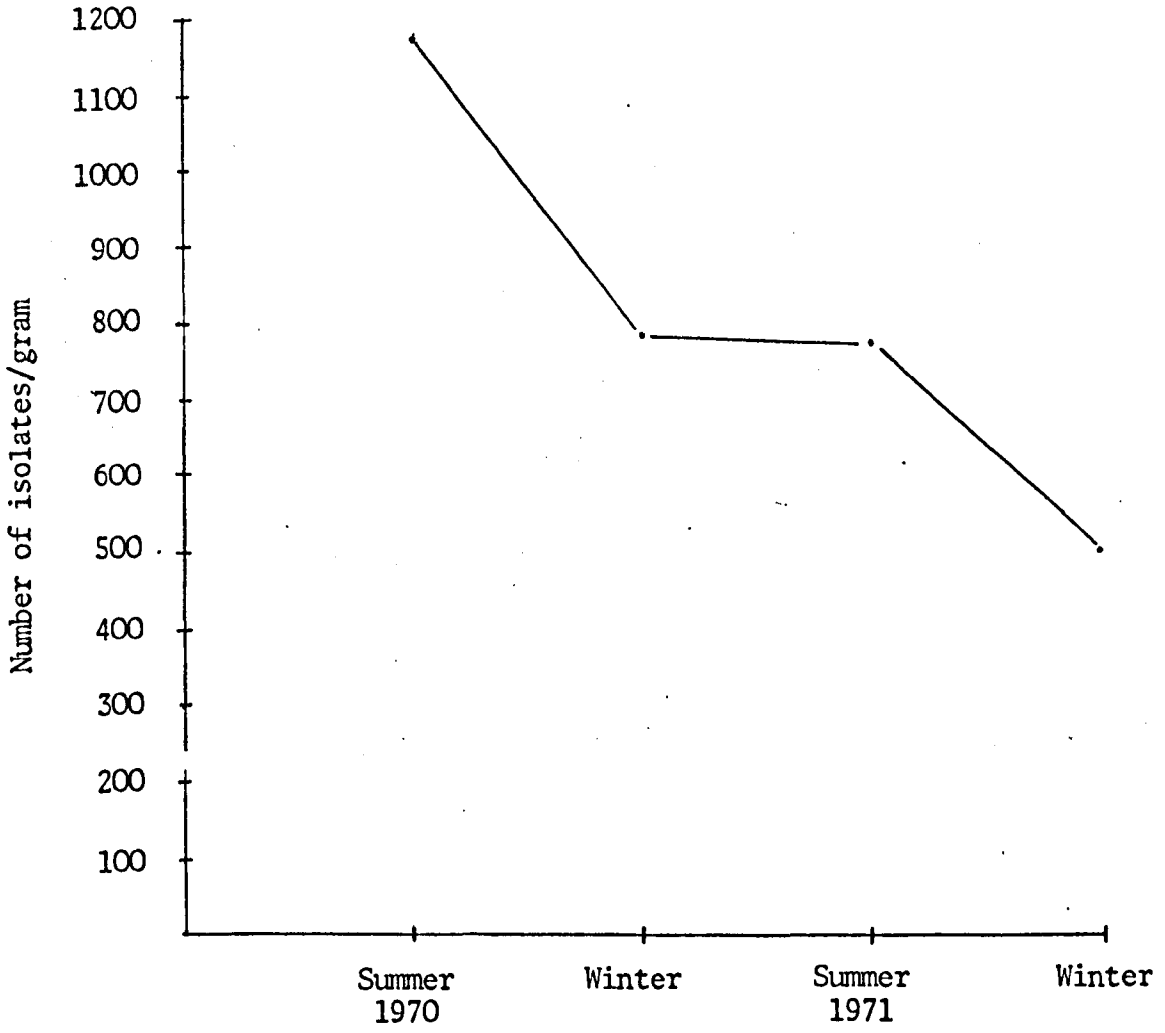


Figure 2.23

Number of isolates/gram from Site 5 during two years

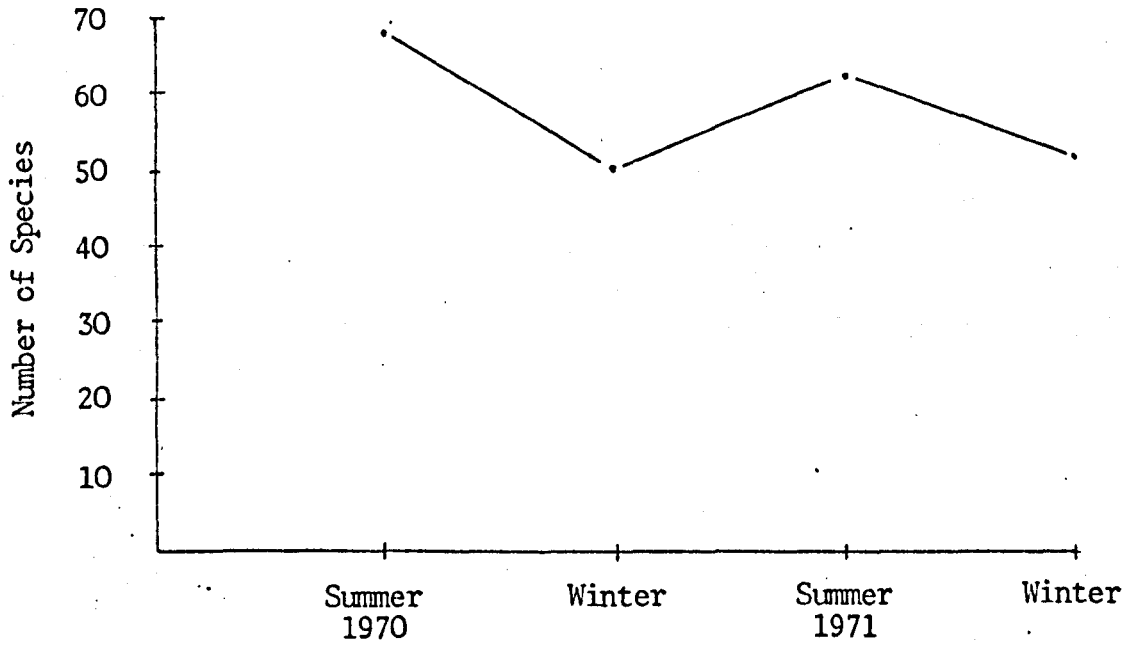


Figure 2.24

Number of species isolated from Site 5 during two years

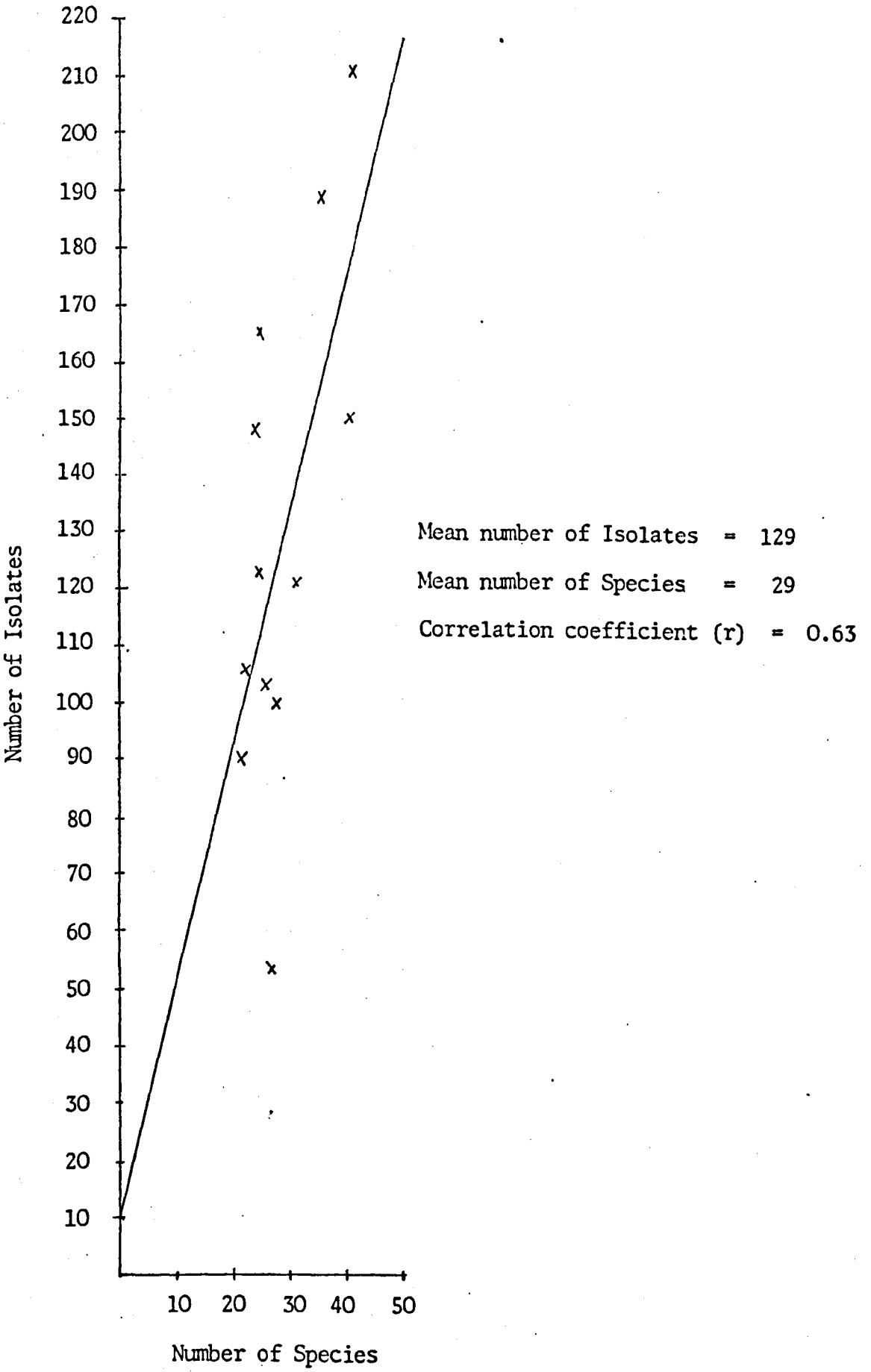


Figure 2.25

Regression line of number of isolates on number of species from Site 5

This indicates that although there is much growth fluctuations during the two years as reflected by the isolates and the species, but the slight relationship suggests that both are responding to the same influences.

Graphical analyses of the effect of season and depth on the numbers of isolates/gram and species are presented in Figs. 2.26 and 2.27. In an arable field the effect of depth is disrupted by ploughing so that the levels are altered each season and the soil is generally lacking in structure. An additional factor is the seasonal variation of fallow followed by the sowing of a crop which grows and is then reaped and the residues usually ploughed in. The numbers of species occurring at different depths generally decrease in number with increasing depth. This is not so marked between the 5cm and 15cm depths which contain a very similar number of species. The topsoil generally contains the largest numbers of species. The top three layers reflect the seasonal variation shown in the total numbers of species. However at 25cm the numbers follow the pattern of the numbers of isolates. It seems likely that the upper layers would be most affected by seasonal variation which is sheltered from the 25cm level, where other factors are involved. It may be that when conditions are good or bad for growth the effect is also apparent at 25cm depth so giving rise to a similar pattern of species to the total numbers of isolates. The lower levels may also be affected by drainage of water from the upper layers.

The numbers of isolates/gram at different depths (Fig. 2.27) show a similar pattern to the total numbers of isolates. There is generally a decrease in the numbers with increasing depth. However, it is difficult to explain the reason for the high numbers of isolates in the first summer at 5cm and 25cm. One would expect similar conditions to be occurring at the other two levels but if they are

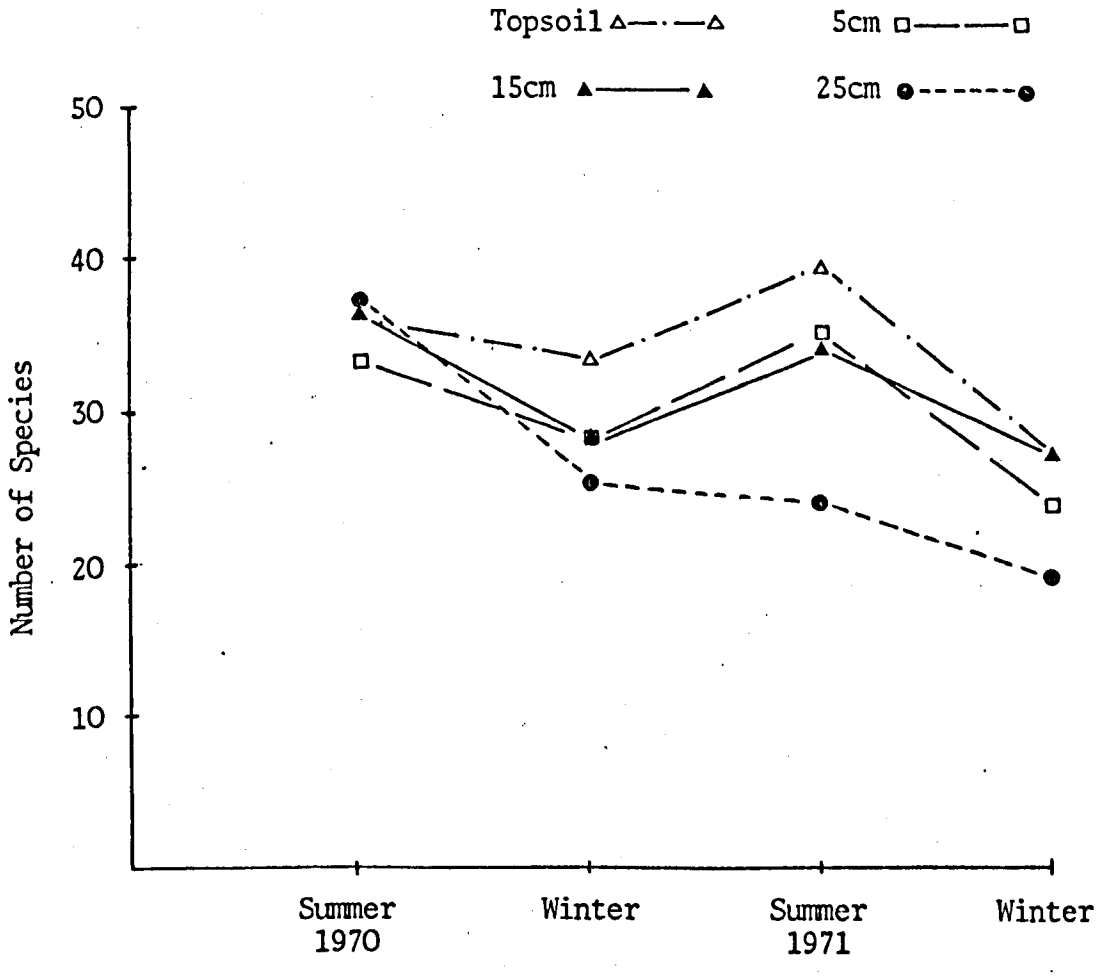


Figure 2.26

Number of species at different depths from Site 5

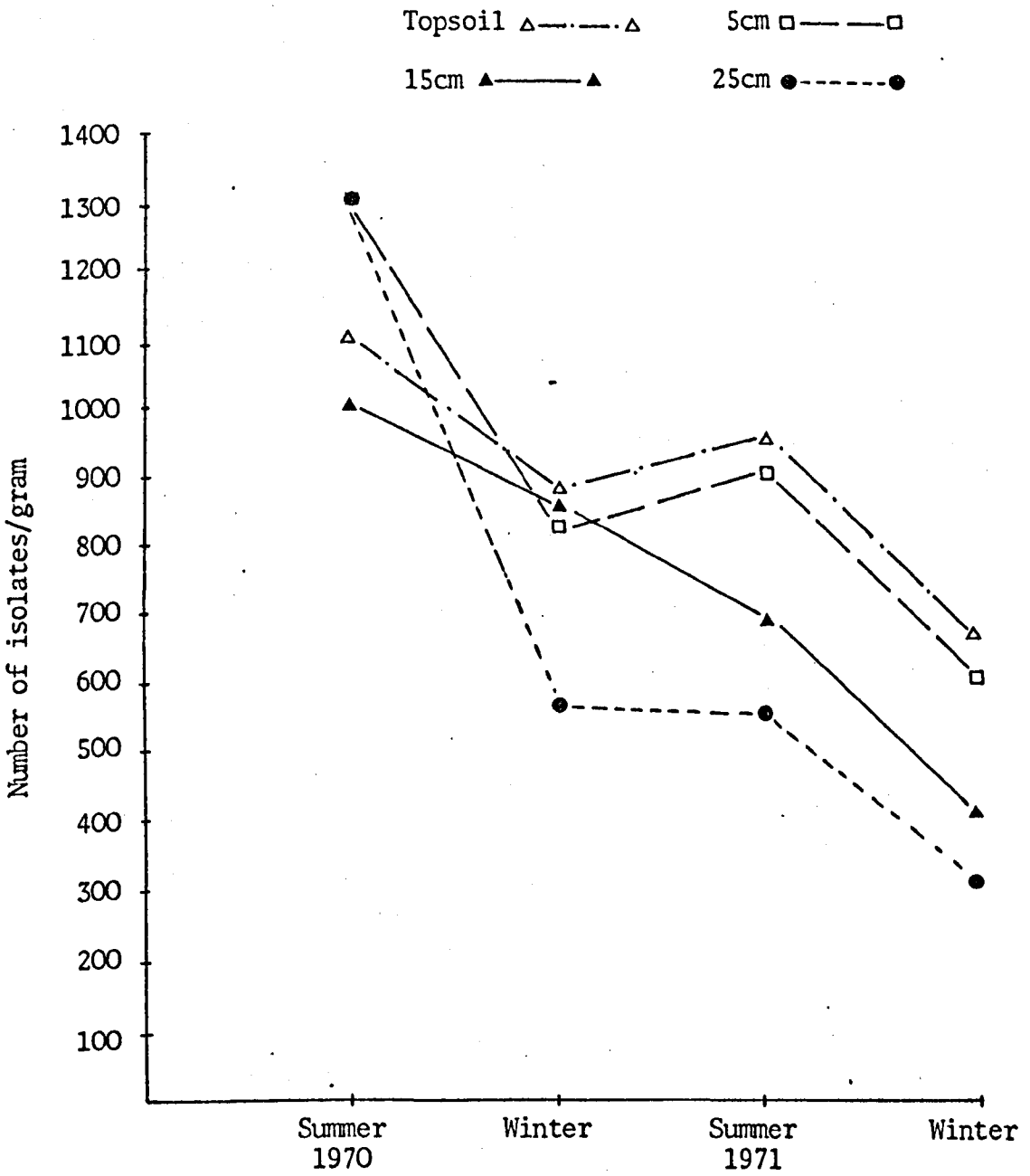


Figure 2.27

Number of isolates/gram at different depths from Site 5

it would seem that the response of the mycoflora is not of the same magnitude.

The environmental factors which were measured at each sampling are presented in Table 75 in the Appendix and summarised in Table 2.9. The pH of the soil is generally alkaline with little alteration in depth. There is some slight seasonal variation in the range but not of a regular pattern. As there is no alteration with depth it is impossible to assess the effect of pH in association with depth. The Summer of 1970 and the Winter of 1971-72 are both slightly more alkaline than the other two seasons but there is no similarity in the quantitative mycoflora so that other factors are involved. The other two seasons are similar in range although the Winter of 1970-71 has a lower extension. These two months contain a similar number of isolates which may be due to some extent by the pH. It is difficult to assess the true role of pH as a direct influence since there is very little variation. It is probably relevant in conjunction with other influences of the environment. The general alkalinity of the readings may have caused some reduction in numbers of isolates as there are relatively few in comparison with other soils.

Warcup (1957) found soil moisture very influential in his study of an Australian wheat field. However, the samples from this present site do not show much variation in soil moisture with depth or with season. There was a slight increase in the first winter which may have influenced the numbers slightly. The soil generally contains a reasonable amount of water, often feeling sticky when sampling, due to the high clay content of the soil, which reduces pore and air space. This probably acts to decrease the numbers at this site particularly when the soil is fallow. This is also

Table 2.9

Average lead content, soil moisture and range of pH of soil samples from Site 5

Samples	Lead content (in p.p.m.)		Topsoil		5cm		15cm		25cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	H_2O	pH	H_2O
Summer 1970	816	2,786	7.3 - 8.5	20	7.5 - 8.5	23	7.3 - 8.5	22	7.2 - 8.5	22
Winter	283	2,733	6.8 - 7.4	31	6.8 - 7.3	30	6.9 - 7.3	32	6.9 - 7.4	29
Summer 1971	600	2,700	7.1 - 7.4	20	7.4 - 7.4	26	7.0 - 7.4	22	7.3 - 7.4	22
Winter	283	1,633	7.6 - 7.9	20	7.3 - 8.1	26	7.3 - 7.9	24	7.2 - 8.0	24

suggested by the fact that on visual examination the soil contains much water. Since there is little variation in the amount of soil moisture it is difficult to trace whether there is any direct relationship between the mycoflora and soil moisture with regard to the different samples.

Although this field has been contaminated to some extent by lead, in comparison with the spoil heaps this is not of any great magnitude. There is some variation in the amount of lead extracted. The Hac extractable lead is markedly less than the total lead content which may be due to the clay content of the soil and that it may be binding some of the lead present. There may also be other structures in the soil capable of binding lead to them. The total lead content is relatively constant with the exception of the second winter sampled which may just be due to some variation in the soil at this sample. The Hac extractable lead is less in the winter months quite markedly. This may be due to an alteration in the structure of the soil while it is fallow so that some of the lead is lost from the more easily extractable positions. There is no marked response by the mycoflora to the decreased lead content but other factors may be involved since the soil is lying fallow and it is winter. The lead content is probably more important in lowering the numbers throughout the sampling period rather than any direct effect causing alterations in the individual samples. The more specific changes are probably influenced strongly by the agricultural practice which may be the paramount factor with the others contributing.

During the two years of sampling 133 different species were isolated in widely varying numbers. Many of the species were irregular in occurrence and when they appeared in the sample it was in low numbers. Many of the species occurred only once during sampling, also many occurred in only one season. This is shown

clearly by the following table:-

Table 2.10

Number of seasons in which a species occurs.

1 season	-	74 species
2 seasons	-	30 species
3 seasons	-	16 species
4 seasons	-	13 species

The importance of these ephemeral species is difficult to assess and should not be ignored, as they may be important but fail to occur in large numbers on soil plates.

There were many Penicillia isolated during the sampling period. The number of species was fairly constant although the same ones were not isolated regularly. There were rather fewer in the first winter sampled. The Penicillia as a group do not show any seasonal variation.

In addition to the Penicillia there was quite a range of Aspergillus species isolated, although most of them occurred in very small numbers and not regularly. Aspergillus fumigatus contributed the most numbers by far, and generally figures for the total numbers reflect the pattern of occurrence of this species. Numbers are usually greater in the first year of sampling. As the crop is growing there is a marked decrease in the numbers of Aspergilli which possibly are not so well suited to conditions during the growth of a crop and consequently suffer while other fungi increase. In March 1971 there is a large number of two species of Aspergillus - Aspergillus chevalieri var. intermedius and Aspergillus glaucus - probably due to the fact that the incubator failed during the incubation of soil plates affecting the results.

In addition to the large number of Fungi Imperfecti there is a considerable number of Phycomycetes especially Mucor and Absidia species, more than occurred in the previous sites. There is marked fluctuation in the numbers isolated over the two years. The occurrence of these fungi is to be expected as they are rapid sugar decomposers and are generally stimulated by green vegetation (Chesters 1949). Warcup (1957) found that there were high numbers during the decomposition of the crop residues. However, there is no definite seasonal pattern of these fungi at this site, although they do tend to increase slightly after cultivation of the crop and they occurred in greater numbers in the second year.

Twelve species were isolated in the four seasons and they were:- Absidia spinosa (66%), Coniothyrium fuckelii (75%) Fusarium culmorum (75%), Fusarium sambucinum var. caeruleum (50%), Gliocladium roseum (83%), Mucor microsporus (50%), Penicillium canescens (66%), Penicillium cyclopium (75%), Penicillium decumbens (58%), Penicllium frequentans (58%), Trichoderma viride (83%) and Verticillium candelabrum (58%). In addition sterile isolates (100%) occurred at all times. The numbers in brackets refer to the number of individual samples in which the fungus occurs. Three other species occur in six samples or more, they are Aspergillus fumigatus (58%), Mucor hiemalis (50%) and Penicillium funiculosum (50%), however they do not occur in all four seasons. It would seem likely that these fungi probably occur in the soil in all four seasons sampled but the numbers in one season were not sufficient for them to be isolated. All these fungi are considered to be normal soil inhabitants of varying importance (Gilman 1957). The five species of Penicillium may be partially indicative of the selective effect of the soil plate method of isolation, but according to Raper and Thom (1949) all are to be

found in differing frequency from soil plates. Three Phycomycetes are isolated fairly regularly during sampling possibly due to stimulation by the surface vegetation and consequently the relatively high amount of simple organic matter available for decomposition.

Coniothyrium fuckelii occurs in low but regular numbers throughout the sampling period. This is interesting as it would seem to indicate that the fungus is only able to exist in this environment and that good growth is not favoured. Similarly Brown (1958) found that it occurred in alkaline sand dunes in the later stages of colonisation which are not a very favourable habitat. Verticillium candelabrum occurs also throughout the sampling period in low numbers. Aspergillus fumigatus is a very common soil inhabitant which occurs in three seasons here and six individual samples, suggesting that it is probably existing in the soil most of the time but not in very great numbers. It seems that these fungi only tolerate the conditions here most of the time, but can take advantage of the periods suitable for their growth.

Two Fusaria occur quite regularly during the sampling period with others occurring less regularly but in quite large numbers. Fusaria are frequently isolated from agricultural soils (Bisby, James and Timonin 1935) and are generally of economic importance as they attack many crops. Fusarium culmorum is particularly associated with brown foot rot and ear blight of cereals. This fungus is a strong saprophyte and is commonly found in poorly drained heavy soils (as this site is) but is generally associated with acid conditions which do not prevail here (Moore 1959). The fungus seems to be stimulated in the summer season which coincides with the growth of the crop and harvesting, also probably enhanced by inoculum ploughed in so that it continues during the winter months. Fusarium sambucinum var. caeruleum

does not occur so regularly as F. culmorum but is a regular inhabitant of the soil occurring at any time showing no seasonal variation. These two species have been known to act together in infections of plants.

The remaining species - Gliocladium roseum and Trichoderma viride and sterile isolates - are all common soil inhabitants. In most soil studies there are large numbers of sterile isolates, some of which are eventually identified as Basidiomycetes. They are probably sterile due to the method and conditions of isolation not being suitable for their sporulation. Gliocladium roseum is an active saprophyte and also can be parasitic on other fungi (Barnett & Lilley 1962). It is generally found in alkaline soils and is often associated with the surface vegetation (Pugh & Dickinson 1965). It generally occurs in large numbers in the summer months while the crop is growing and just after reaping. Trichoderma viride is also a common soil fungus having been isolated from many varied soils in different investigations (Barron 1968), it generally occurs in established soil associated with the surface vegetation (Bridge Cooke & Lawrence 1959). Both fungi occur in fairly large numbers particularly the latter and both show variation during the sampling period. Peak numbers generally occur during the growth of the crop which may be due to the organic debris from the crop which would be available for increased growth. The numbers decline in the winter months during the fallow period which argues in favour of the probable effect of the surface vegetation. They are also known to occur in large numbers in the rhizospheres of plants.

Considering the density of the species there are only three which contribute more than 5% of the total isolates, this is in marked contrast to the relatively large number of species which

occurred regularly. The three species are:- Aspergillus fumigatus, Gliocladium roseum and Trichoderma viride, all of which are common soil inhabitants and known to influence the mycoflora of soils strongly. Sterile isolates also contribute more than 5% of the isolations. Excluding the sterile isolates all of these species are favoured by the soil plate method as they are quite heavy sporing and relatively fast growing which may influence their numbers investing them with a relative importance which may not be so in the soil. Aspergillus fumigatus occurs less regularly than the other species and yet it contributes a large number of isolates. This may be due to its ability to make the maximum use of optimum conditions when they occur so that it gives rise to large numbers when the environmental conditions are suitable for its growth. The other two species show increases which may reflect the changing environment being sometimes more suitable for a particular fungus.

In the consideration of the qualitative effect of depth the disruption of the horizons and changing conditions due to agricultural practises must be considered. As the layers are regularly reversed it would seem likely that there would not be such a marked effect of depth and this is so as the numbers of species and isolates are similar in the four levels. There are slightly more isolates in the top two layers and more species in the topsoil. This is probably a result of the inoculum from the air and also the immediate supply of organic material when the Barley crop is sown. Many species occur only occasionally and are not present in all the horizons. Such isolations may be due to the chance spore, or the sampling method may not encourage these species so that no conclusions can really be drawn about these species. However the fungi which occur in larger numbers generally occur at all depths showing little or no preference. Some

of the Phycomycetes show a preference for the upper layers particularly Mucor microsporus, which is probably associated with their dependence on relatively simple organic substances which would occur in greater amounts in the upper layers. However they do occur at all levels with Mucor hiemalis showing a slight increase at 25cm which may be associated with the reversal of depth by ploughing which it may be better able to withstand than the other Phycomycetes.

Penicillium species generally occur at all levels although there are many species which are isolated only occasionally. Many of these are to be found in the top two layers and may be the result of some spores being deposited from the air. Fusarium species also occur at each level sampled generally in similar amounts, showing no preference possibly because of the ploughing altering the levels and their strong saprophytic growth. Aspergillus fumigatus and Gliocladium roseum both show a tendency to occur in larger numbers at the lower levels. This may be due to their ability to withstand the conditions prevailing at the lower depths more efficiently than the other fungi unlike Trichoderma viride which occurs mainly in the topsoil and at 5cm depth, which may be due to its close association with plants growing in soils and its rapid growth while the plant grows. These are general trends shown by the fungi over the two years sampling and they may not coincide with the position of the fungus in each sample.

There is a wide variety of species isolated from this site but it is limited in comparison with other studies using the same method. The main difference between this field and other agricultural soils is the contamination by the lead which is probably an important factor in lowering numbers. However, the form of agriculture practised is of paramount importance in influencing the mycoflora within the field throughout the sampling period.

CHAPTER 3

GENERAL DISCUSSION

The following table summarises the history and type of soil for each of the Sites investigated. The spoil heaps all date from pre 1940.

Table 3.1

History and soil type of the five Sites

SITE	HISTORY	SOIL TYPE
1	Untouched waste from smelting process	Coarse topsoil - calcite fragments - with sandy lower layers.
2	Untouched waste from smelting process	Similar to Site 1, but sandy throughout profile.
3	'Tailings' with topsoil added (date unknown)	Sandy profile with added topsoil just discernable - darker in colour.
4	'Tailings' with topsoil added (December 1970)	Added topsoil - agricultural loam, lower layers coarse mine waste.
5	Agricultural field	Clay to all depths sampled.

Burges (1963) discussed some of the problems which confront a soil microbiologist, one of which is the sampling procedure. The soil is a very heterogeneous environment and this is particularly so in spoil heaps where the nature of the spoil may alter markedly over short distances, the changes being such that samples taken only 10 cm apart may show large differences. Since a pit is dug during a

sampling in this investigation the same spot cannot be sampled again, for even if the pit were carefully filled the material has been disturbed and so the results would be affected. Consequently each sample was taken from as small an area as possible to try to avoid large scale changes, although this cannot be guaranteed. The heterogeneity of the soil was demonstrated in the preliminary study (Section 2.2) when a number of samples of topsoil were taken from each site, and it was found that several samples were necessary to isolate all the species present.

In addition to taking samples from the same spot, the soil should ideally be sampled daily to ascertain small scale changes in the fungal population. The study should be accompanied by an investigation of the environmental factors which may also vary from day to day and affect the mycoflora. This would give a reasonably accurate picture of the soil flora. It is not feasible to take samples daily and it was decided to sample every two months for two years in order to examine long-term changes and variation. However, although the soils were sampled at the same time each year the seasonal variations may not be the same in successive years, and this may account for some sample variation. As there is no distinct seasonal occurrence, such as leaf fall at this site, it was thought that this method of sampling would be the most satisfactory that could be conducted. It would not be feasible for an individual to take sufficient soil samples from the different levels of the five sites to gain a true picture of the mycoflora at each sampling time as the number of plates necessary is very high (Chapter 2, Section 2.2). Owing to this the results obtained from individual samples have been added together in groups of three to try and reduce random variation.

Another factor which has considerable influence on the results

is the method of isolation, as it has been shown that different methods can often produce different results (Chesters & Thornton 1956). In this investigation the soil plate method of Warcup (1950) was used with 50mg of soil for four sites and 10mg for the other site which was comparatively heavily populated. The number of fungi may be influenced by the number of plates examined and up to a point the more plates examined the greater the yield of species (Burges 1963). The numbers are also affected by the varied ability of fungi to grow and compete with other fungi on the soil plate (Dwividi & Garrett 1968). This means that fast growing, heavily sporing fungi and those which produce fungistatic substances have an advantage and may dominate the plate so that the slower growing fungi may not be isolated. Warcup (1957) said that the effect of soil plates on an investigation was that they "tend to favour medium to fast-growing fungi present in soil in relatively low numbers."

However, one of the other problems caused by the soil plate is a very important one. The soil plate method gives no clue as to the origin of the colonies on the plates (Warcup 1955), which may be derived from either spores or hyphae, although the latter is not generally considered to result in many colonies on the soil plates. This means that an assessment of the activity of the fungi in the soil using soil plates is very difficult. Active fungi at the time of sampling would be those which were mycelial in form, although some spores may have just been produced and are in a sense part of the active growth of a fungus at that time. However, most spores are the result of activity which has occurred at some time in the past, although the actual time of activity may be difficult to ascertain. With regard to the topsoil fraction the spores which appear on the soil plate may have been produced elsewhere and blown in by the wind.

This is not so likely at the lower levels, as only a few spores are washed down from the surface (Burges 1950).

The most efficient method of assessing the activity of the soil population is by visual examination of the soil by such methods as that of Jones and Mollison (1948). Despite examination of a larger volume of soil to increase the likelihood of mycelium occurring in the sample, this method proved unsuccessful in the present investigation. Since most isolates on soil plates arise from spores and since the numbers of isolates was very low it is only to be expected that the likelihood of seeing mycelium in a relatively small amount of soil would be minimal. The limitations of the soil plate method must therefore be remembered when considering the results and an investigation using other methods may produce different results (Warcup 1957).

In addition to the method of isolation there are other factors which may influence the results. The medium of isolation can affect the fungi which are isolated and this has been used to good advantage when isolating certain groups of fungi which utilise certain substances more efficiently than most other fungi. Potato dextrose agar was used since it proved to be the least selective agar tested. Similarly, Rose Bengal which was used to decrease bacterial contamination and to lessen the growth of the fast spreading fungi may affect the results. This solution was found to be more efficient than various antibiotics tested and it was not so selective as strong acidification which was another alternative. The effects of adding Rose Bengal to the medium were discussed by Smith and Dawson (1944).

Apart from the isolation method and the media used the temperature of incubation may influence the results. Temperature affects all the activities of fungi which generally have an optimum

temperature range in which they grow most efficiently and if the incubation temperature is within this range some fungi may have an advantage over other fungi whose optimum range does not include the temperature of incubation. Consequently some fungi living in soil may not be isolated, whereas others may be isolated in disproportionate numbers. The fact that the temperature of incubation affects the results of a soil study was shown in March 1971 during this investigation. In this month the heating system failed over the first 24 hours of incubation lowering the temperature to $0^{o}C$. As a result, there were fewer fungi isolated than normally and two species occurred in relatively large numbers which were not found in other samples. The incubation temperature used in this investigation was $25^{o}C$ which is much higher than the general soil temperatures, however, it is a general laboratory temperature and was used to speed up development of colonies but it may of course have influenced the results.

These are the general factors attendant on the isolation procedure used in this investigation which may influence the results of the sampling. There is one other factor which has influenced the results of this particular investigation and that is that half of the soil plates contained lead as lead nitrate at a concentration of 400 p.p.m. Owing to the large numbers of plates being dealt with it was not possible to make up agar individually for each plate. The agar and lead solution was swirled as much as possible to facilitate mixing. However, lead does not mix very well with agar and an even dispersion throughout all the plates cannot be guaranteed, consequently there may be some variation between plates.

In general there were fewer isolates and species present on the lead-containing plates in the individual samples. It is to be expected that the numbers would be less as lead is a toxic substance

and development of fungi on such plates would be inhibited or possibly in some cases completely prevented. Nevertheless, it seems that some species are able to grow and so are isolated from the lead-containing plates, e.g. Aspergillus fumigatus. The amount of lead was therefore not totally prohibitive but growth is slower and some species may not grow at all.

Table 3.2

Relative Percentages of Species from each site on the different soil plates

SITE	LEAD STATUS*	P.D.A.	P.D.A. + Pb.	BOTH
3	1	46	21	33
2	2	35	26	39
1	3	48	20	32
4	4	38	18	44
5	5	35	19	46

* 1 is HIGHEST, 5 is LOWEST

However, when the results are considered in total for the two years the presence of lead does not seem prohibitive since more than half the species isolated at each site were found on lead-containing plates. This indicates that many species can tolerate lead in the agar. However, there were also a large number of species which were never isolated on lead-containing plates. Even those species isolated on both types of plate grew more profusely on the lead-free plates. There seems to be little indication of any relationship between the lead status of the spoil and the percentage of species tolerating

lead. Some species occurred only on the soil plates containing lead and this may follow the reduction in numbers on the lead plates which effectively would decrease the competition.

However, when all the sites are taken together there are 29 fungi which occurred only on lead-containing plates throughout the investigation. Some occur at more than one site - Penicillium corymbiferum, Pestalotia pezizoides, Sporotrichum chlorinum - but even these do not occur in any numbers or very often. As these 29 species are of such occasional occurrence it is difficult to assess their importance in the soil. They may be active members of the soil population which are only able to develop on the lead plates where there is less competition than on the agar plates. However, they may just be the result of a chance occurrence and as such of dubious significance. The fungi which comprise most of the isolations are generally found on both types of soil plate occurring in greater numbers on the plates without lead.

Quantitatively there are far fewer isolates in these soils than are generally found in normal soil. The wide variety of soils

Table 3.3

Average Number of Isolates/Gram at each Site

Site 1	56 isolates/gram
Site 2	48 isolates/gram
Site 3	72 isolates/gram
Site 4	181 isolates/gram
Site 5	811 isolates/gram

of soils studied by Jensen (1931) contained from 24,000 to 460,000 fungi/gram soil using a dilution plate method. Even Site 5 which

is quite good agricultural land only contains an average of 811 isolates/gram which is very low. Bisby, James and Timonin (1933) using a dilution plate method found that the number of fungi in the top four inches of an untreated wheat field soil was never less than 25,000/gram. Site 4 also has slightly more isolates/gram than the other sites, which is probably due to the addition of 'clean' topsoil which introduced a more favourable environment and increased inoculum. The other three sites contain a similar number of isolates/gram which is exceptionally low. The low numbers in Sites 1, 2, 3 is probably due to the poverty of the spoil material as there is little organic matter to act as nutrients for the mycoflora. Site 3 has slightly more isolates/gram than Sites 1 and 2 and this may be due to its age so that it may have had longer to develop and may now be a more suitable environment and more varied than Sites 1 and 2. Site 5 (the agricultural field) has a relatively high number of isolates/gram possibly resulting from the comparative richness of the soil in organic matter.

There is another factor which is probably important and that is the lead content (Table 3.2) of the soils at the different sites. Site 5 contains much less lead than the other sites, followed by Site 4 after the addition of topsoil and these two contain relatively more isolates/gram. However, Site 3 contains a large amount of lead when compared with the other sites and therefore would not be expected to contain more isolates/gram than Sites 1 and 2. At some time in the past (date uncertain) Site 3 has had some topsoil added which has been absorbed into the spoil, and probably exerted a beneficial effect at the time. It may have left a residue of inoculum and also provided time for the establishment of fungi. It would seem, that the lead content of the soils has an important effect on the numbers of

isolates/gram, but that organic material might counteract the lead to some extent. Several studies have shown that even in very inhospitable conditions fungi can actively grow, provided there is a supply of organic material (Brown 1958, Pugh 1962).

A statistical analysis was conducted on the topsoil fraction of Sites 1, 2, 3 and 5. Site 4 was omitted because it is difficult to relate to the other sites as the deposition of additional topsoil, buried the original and so upset direct comparison. The lower levels were not analysed as no fungi were isolated from some samples. An analysis of variance was carried out which showed that Sites 1, 2 and 3 were very similar in numbers but that Site 5 was significantly different from the other sites in the topsoil fraction. This may be the case if all the soil was considered, as the topsoil fraction generally makes up a large and influential part of the total results.

The major difference between these soils and normal soils is the lead content. Sites 4 and 5 have much less lead in their soils and generally contain more isolates in each sample throughout the sampling period. In Site 5 this may be due to the soil being richer in organic matter, and in Site 4 the addition of topsoil during the sampling period and previous attempts at landscaping the area, effectively reduced the amount of lead in the soil. The other sites have little in their favour to develop a thriving mycoflora as there is practically no surface vegetation to supply nutrients with which fungi seem able to withstand fairly intolerable conditions. Site 3 has at some time had topsoil added for landscaping purposes and this may be the reason why in spite of a higher lead content it has a relatively large number of isolates/gram, in comparison with Sites 1 and 2, although their organic content is similar.

Only the lead extracted by acetic acid is thought to be

active biologically, whereas the lead extracted by Hydrofluoric and Hydrochloric acids is the total lead contained in the soil analysed. The former is less than the latter as it usually consists of the more easily exchangeable ions such as those which occur on the surface of soil colloids. The HF/HCl-extracted lead would be made up of the exchangeable ions plus the lead which was more tightly bound into the structure. The amount of biologically active lead is influenced by the fact that many lead salts are insoluble and consequently there may be little lead in the soil solution. Although rain is usually slightly acidic and dissolves many substances it will not in all probability affect the available lead content. Sites 1 and 2 do not show marked differences between the amounts of lead extracted by the two methods and this may reflect the lack of structure in these soils as there is nothing to bind the lead tightly. In Site 4 the two amounts of lead differ markedly, both having been reduced by the addition of 'clean' topsoil, which may have acted as a diluent apparently reducing the lead content. In samples from Site 5 there is some variation in the difference between the two amounts of lead. These differences may reflect the state of the soil colloids at the time of sampling. In some cases they may bind much of the lead while in others the amount may be reduced. Site 3 shows very marked differences in the amount of lead from each extraction procedure. This may be due to the fact that this spoil heap consists of fairly old 'tailings' in which some lead may still be held in the tight combination in which it was laid down underground and the lead which is easily extractable may be that which has been loosened by the working of the material for the ore or been brought into solution at some time and is now held loosely. This site contains much more lead than the others, possibly due to method of production.

At none of the sites was there any direct correlation between variation in the lead content and the numbers of isolates. In Sites 1, 2 and 3 there was little variation in the amount of lead extracted by acetic acid. There was, however, some variation in the numbers of isolates which does not indicate any reaction to the slight changes in lead content. At Sites 4 and 5 there were some marked differences in the Hac extracted lead. At Site 4 the soil samples in Summer 1970 contained more lead than the following samples. This was before the addition of topsoil so that the sample consisted only of 'tailings'. There were fewer isolates/gram in this sample, this may in part be due to the higher lead content but may also be due to the fact that the topsoil had not yet been added, bringing with it inoculum and a healthy environment. In Site 5 also the soil sampled in the first summer contained more lead than later samples. But in this site the soil also contained the most isolates/gram.

Consequently it would seem that the lead content does not directly affect the numbers of isolates causing short term variation. However, the low numbers of isolates found in these sites may be due to the presence of lead.

It is difficult to ascertain how many of the fungi isolated during sampling actually grow in the soil. However, assuming that some of them are able to grow actively in the soils under investigation it would seem unlikely that they are actually in contact with the high levels of lead extracted, and they may be restricted to local areas of soil where the lead content is low. Even if there is lead in the immediate vicinity of the fungus it may not be in a form which is active against fungal growth, but may be combined with minerals

in the soil or be largely in an insoluble state and it is from the soil water that most substances are taken up for use by organisms of the soil. With the exception of lead nitrate most of the lead salts likely to occur in soil are insoluble.

In November 1971 the soil was analysed for lead content using the acetic acid extraction at each level at each site in order to examine whether there was any relationship between lead and the number of isolates. Further, the sites could be compared as the seasonal factors would be similar. Figure 3.1 shows the lead content and the numbers of isolates at different depths in the different sites. There does not seem to be any direct relationship between the amount of lead at a particular depth and the number of isolates at that depth, although clearly when there is less lead there is an increase in the number of isolates. There is much variation between the levels and their lead content in Sites 1 and 2. In Site 1 the lower levels contain more lead than the top two layers and in Site 2 the topsoil contains markedly more lead than the lower levels. It is difficult to say whether the low numbers at 15cm and 25cm depth at Site 1 are due to the higher lead content or other factors, such as lack of organic material or variation in the air and soil moisture content. It would seem more likely to be the effect of depth since the 5cm level contains much less lead, but also has fewer isolates which would not be the case if the lead was the major influence. Site 2 also seems to reflect the fact that there are other influences which act on the mycoflora since the layer with the most lead also has the greatest number of isolates, probably due to it being the topsoil and so the most hospitable layer in a new substrate. The lower levels are similar in lead content and two of them contain a similar number of isolates, but it would seem that at 15cm there is some factor

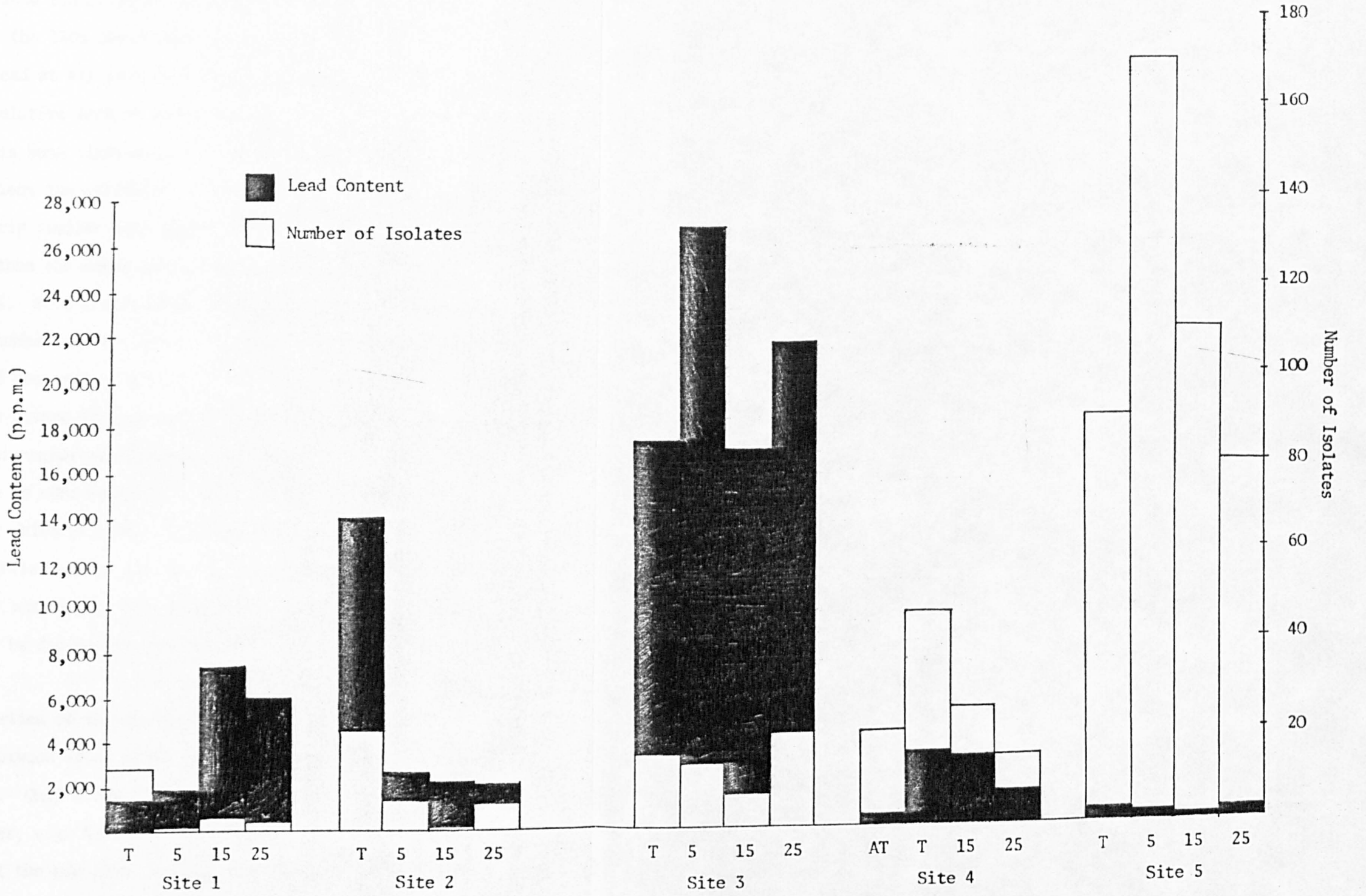


Figure 3.1

Lead Content (Hac extracted) and number of Isolates at different depths at the five Sites in November 1971

other than the lead rendering it an unfavourable place for fungal development, as the 25cm layer contains several fungi. Site 3 contains more lead at all levels than any other site, but this has not lead to a relative lack of number of isolates at the different depths. There is some fluctuation in the numbers of isolates, but it does not reflect the variation in the lead content. In fact the numbers are fairly similar with the exception of 15cm depth which has fewer isolates than the other layers while having less lead than the other depths. Site 4 also shows little reflection of the lead content on the numbers of isolates. The added topsoil which contains very little lead does not give rise to the largest number of isolates whereas both the former topsoil and the now 15cm level contain more lead and a larger number of isolates, particularly the former topsoil which may be due to some beneficial effect of the added topsoil in addition to that which affected the former topsoil in July. Site 5 contains very little lead at any level, but the numbers of isolates do vary with the 5cm sample containing more isolates than the other levels which may be due to the residues from the crop which has been ploughed in.

The comparison of the numbers of isolates at different or similar depths between sites shows little direct relationship to the lead content. Only a few layers contain similar amounts of lead at different sites, e.g. Site 1 at 5cm and Site 2 at 25cm, Site 2 at 5cm and Site 4 at the now 15cm, and the added topsoil of Site 4 and the topsoil of Site 5. The first two pairs are difficult to assess as they are from different depths and so there is variation in the factors affecting the soil population. Site 1 contains fewer isolates in the 5cm fraction than the 25cm fraction of Site 2. In fact the 5cm level of Site 2, which contains more lead than the equivalent layer at Site 1, also contains more isolates. So that it would

seem that Site 2 is more hospitable in this sample than Site 1, even at depth, although the numbers involved are very low. In a comparison between the 5cm layer at Site 2 and the now 15cm layer at Site 4 the number of isolates shows a distinct difference, as there are more isolates in Site 4. This may be due to the influence of the added topsoil in providing inoculum and organic supply in the form of plant cover whose roots would penetrate to this depth and would possibly be dying down at this season. Site 2 has none of these advantages and so the number of isolates is very low. The other pair show a very marked difference in the numbers of isolates ranging from 90 in the topsoil at Site 5 to 21 in the added topsoil at Site 4. Site 5 is an almost normal soil differing in the fact that it contains lead but it is obviously a much more favourable environment than Site 4 added topsoil, which itself is not a true part of the spoil heap and would be expected to compare reasonably well. In November there was surface vegetation at Site 4, but Site 5 was laying fallow at this time. The organic content of Site 5 is probably higher since the whole profile has been farmed for a long time. Site 4 topsoil was added in December 1970 and there was no replacement of any materials removed so that it is probably poorer. It is this factor rather than the lead content which is affecting both sites, although the higher numbers of isolates when compared with the other sites may be the result of the lower lead content.

It would seem that there are factors other than lead which are of considerable importance in influencing the numbers which occur, such as the seasonal factors of climate, the amount of organic content and the general feature that increased depth is not a beneficial environment for fungi. The high lead content of Site 3 does not result in very low numbers of isolates for this site as would be expected,

as in comparison with Sites 1 and 2 the numbers are relatively high. This feature is difficult to explain except that there has been attempts at landscaping by planting trees and adding topsoil at some time which may have exerted a beneficial effect which still shows.

Two other environmental factors were measured which may affect the soil mycoflora. The first is the pH which in the soils studied was slightly alkaline. Fungi can generally tolerate quite a large range of pH except during spore germination, but are usually considered more tolerant of acid conditions. However, the gross soil pH measured may not be the actual pH in which the fungus is living as conditions in the soil may vary locally and are constantly fluctuating. The pH of the soil solution, which generally supplies many of the fungal nutrients, may alter the solubility of some minerals necessary for fungal growth. The activity of the fungus itself may affect the surrounding pH.

Several soil studies have shown that the fungal population is influenced by pH. Warcup (1951) examined five similar soils which differed in their hydrogen ion concentration and found that the acid soils contained more isolates and species than the alkaline ones. Even the slightly alkaline soil (which was similar to the pH of the present soils) was not so rich in isolates and species. Warcup also found a qualitative difference between the different soils with some species limited to only one type of soil. The fact that pH was a major influence on the mycoflora was shown by Brown (1958) who studied acid and alkaline dune systems and isolated many of the same fungi as Warcup on soils of similar pH. She also found a distinct flora at the two types of site. Parkinson and Balasooriya (1967) showed that there was a change in the soil population which corresponded

with an alteration in the pH over a short distance in the soil profile. However, Jensen (1931) examined a wide range of soils and could find no correlation between the numbers of fungi and the pH of the soils.

It would seem, though, that pH is generally considered to affect the fungal population particularly the numbers isolated and may influence the fungal species which are isolated from these soils. It is difficult to say how important this factor is since the sites are contaminated with lead and are in general very poor, with the exception of Site 5 which does not contain so much lead and is an agricultural field and so a more favourable environment. The range of pH at all sites shows little variation with season or depth. The range may slightly increase or decrease but it generally tends to be alkaline. There is no correlation of the numbers of isolates with the slight changes in the range of pH during the two years. It may be that the changes are not extreme enough to act on the flora in any way. The general alkalinity of the spoil may have influenced the total numbers of isolates in conjunction with other factors so giving rise to the lowness of numbers.

The second environmental factor which was studied was the soil moisture content. The effect of soil water on the mycoflora is a complex process which has been reviewed by Griffin (1972). It is an important component of the soil containing many of the solutes necessary for fungal growth. However, if the amount of water in the soil decreases it becomes progressively harder for the fungus to exert sufficient suction pressure to empty pores of certain size of their content (Gray and Williams 1971). This may not only decrease the numbers of fungi in the soil but may also affect the mycoflora qualitatively leading to the domination of the environment by Aspergillus and Penicillium species (Chen and Griffin 1966). However,

these extreme conditions associated with drought do not usually occur in temperate regions.

Generally the soil moisture is believed to exert its major effect through altering the amount of air present in the soil which affects the growth of fungi as they are mainly aerobic. Griffin (1966) suggests that it is only in damp soils at lower levels where the pores are not in direct contact with the atmosphere that the O_2/CO_2 balance may be markedly changed so affecting the soil population. The ratio of moisture to air is constantly changing in the soil and may vary considerably on a micro-scale quite often as a result of microbial activity. The main supply of soil moisture comes from the rainfall which fluctuates throughout the year with some months varying by quite a large amount from the average, however, there is little similarity between the gross monthly rainfall and the soil moisture.

Sites 2, 3 and 5 generally do not show much variation in soil moisture either seasonally or with depth. The lower levels of Site 1 also show little variation, probably due to the protection of the surface layers. The sites vary in their ability to hold water with some topsoils containing quite a lot despite the drying influence of the wind and sun. Site 1 has a large coarse fraction in the upper layers which do not hold much water unlike Site 2 which has uniform water holding capacity throughout the profile. Site 5 generally contains the most moisture due to the high amount of clay in the soil, unlike Site 3 which is quite sandy in texture and contains less moisture. There is some variation in the soil at Site 4. The added topsoil generally contains more moisture than the lower levels probably due to its finer texture and greater binding power. In the first winter the topsoil contained a higher amount of moisture

which may be due to its being on the surface in one sample and then covered by the added soil which may have affected the balance of water.

Little correlation was discovered between soil moisture content and the actual number of isolates at different sites. Site 2 where soil moisture varied very little did not have a constant number of fungi indicating that other factors are also important. In the other sites where the amount of moisture does fluctuate there is no regular relationship. Similarly the amount of rainfall for the whole month is not the major influence on the amount of soil moisture present at the time of sampling, but rather whether any precipitation has fallen immediately prior to sampling. It seems that the range of soil moisture content is insufficient to affect the soil population, although it may act in some way to influence the fungal population indirectly.

There does not seem to be any regular seasonal variation at the sites studied and the literature on this topic is contradictory. It would seem likely that Site 5 (the agricultural field) would show a seasonal pattern which might be more associated with seasonal changes in the growth of the crop. However, Gams and Domsch (1969) could find no seasonal variation in a wheat field soil and the variation in Warcup's study on a similar soil was probably due to a summer drought. In this study there does not seem to be any regular seasonal variation in Sites 1 and 5. This is also true for Site 3 which showed an increase in both summer and winter of the second year. At Site 4 the numbers of isolates and species increased after the addition of topsoil so complicating the results. There was a slight increase in the second summer which may be a seasonal response but also associated with the added soil. Site 2 shows a slight increase in numbers of isolates/gram in the summer months.

These are merely trends, as there does not seem to be any great alteration in numbers. This was confirmed using the analysis of variance on topsoil fractions of Sites 1, 2, 3 and 5, which usually closely followed the total results, and showed that there were no significant seasonal differences, indicating that any variation is the result of other factors. This is understandable from the viewpoint that season affects the soil population not directly but through its influence on the surface vegetation as it affects the supply of nutrients to the mycoflora (Eggleton 1938). Tresner et al. (1954) also found that seasonal variation may be associated with changes in other factors such as moisture and organic content. Since there is little or no vegetation on Sites 1, 2 and 3 then it is logical that there should be no evidence of seasonal variation if surface vegetation is the major influence.

It would seem that it is a combination of physical factors which affect the mycoflora of these soils rather than any one factor dominating. Neither pH nor soil moisture were shown to have any direct effect on the mycoflora but they are factors which are involved in the micro-environment in which fungi grow. The lead content also did not have any direct effect with regard to short-term changes but its presence in the soils is probably very important affecting the mycoflora quantitatively. A small quantity of lead in an apparently otherwise normal soil (Site 5) reduced the numbers markedly. After the addition of a normal soil at Site 4 there was an increase in the numbers of isolates. This may be due to the increased inoculum in a suitable environment but the increase also occurred at the lower levels. The cause of this may be inoculum moving from the topsoil associated with the movement of nutrients from the topsoil and the surface vegetation which developed. These two sites have two factors

in common, they are the occurrence of surface vegetation at some time in the year supplying organic matter and a lower Hac extractable lead content in comparison with the other sites. The other three sites contain markedly fewer isolates, a higher lead content and very little organic matter with sparse surface vegetation.

Four of the sites follow a pattern of increasing lead content with decreasing numbers of isolates. The one exception is Site 3 which contains the highest amount of lead and produces more isolates than Sites 1 and 2. There seems some indication that relative amounts of lead have a comparative effect on the numbers of isolates but in some circumstances the numbers can be increased.

Table 3.4

The average number of isolates/gram, lead status and organic content of the five sites

SITE	LEAD STATUS	ORGANIC CONTENT	ISOLATES/ GRAM
3	1	7%	72
2	2	8%	48
1	3	7%	56
4	4	13%	181
5	5	18%	811

The other difference between the sites is the organic content which was measured on the topsoil in the preliminary study. Both Sites 4 and 5 contain more than the other three sites with most organic matter occurring in Site 5. Both these sites contain more isolates/gram, so it would seem that these sites are more suitable through higher organic content and less lead. The organic content would seem to be the probable reason for the increased numbers at the

lower levels in Site 4 which did contain a fairly high content of lead. The other three sites contained a similar amount of organic matter in their topsoils so that it is not an explanation for the higher numbers of isolates at Site 3. Site 3 is made up of natural waste from mining - the 'tailings' from the mine - while Sites 1 and 2 are made up end-products of the chemicals used to clean the furnaces - calcium sulphate, calcium sulphite and calcium oxide. It may be that Site 3 is more conducive to fungal colonisation than Sites 1 and 2. Another factor which may have influenced the numbers in Site 3 is the addition of topsoil at sometime. While this no longer gives rise to an increased organic content it may have provided a 'healthy' environment for a time before it became absorbed into the soil of the site. This may have allowed some fungi to become established to varying degrees and now some are still able to grow to some extent at the site and so give rise to more isolates/gram. It may have also allowed a few species to become well adapted so that they grow in large numbers. It may be that organic content may influence the numbers of isolates which can grow in the presence of lead since fungi can tolerate fairly harsh conditions if there is organic matter present. These two factors are interrelated in acting on the mycoflora and other factors may also be involved. To examine these factors more closely the soil should be sampled more often and also the various environmental factors should be studied on a small scale to try and estimate the actual pattern of existence in the soil between the mycoflora and its environment.

In this investigation samples were taken at several depths in each site. These samples were taken to examine the extent of colonisation of the soils since colonisation of all the levels is usually associated with the environment becoming more favourable, especially

in terms of food supply. Bridge Cooke and Lawrence (1959) found that fungi were isolated at lower levels as the vascular community developed. If colonisation of the lower levels had occurred it was hoped to examine whether there had been any development of a mycoflora different from the surface. It is generally considered that the numbers of fungi decrease with increasing depth (Timonin 1935). This is due in some soils to the lack of organic matter, but this may not always be the main reason since Stenton (1953) showed that there was a decrease in the numbers of fungi with depth in Wicken Fen where it is unlikely to be due to the lack of organic matter. Another feature which is probably influential is the composition of the soil atmosphere. As a result of the activity of the soil organisms the soil air usually contains proportionally more carbon dioxide than the atmosphere. In the upper layers of soil the carbon dioxide is constantly being given off to the atmosphere, but this is not so easy from the lower layers, consequently there is a build up of carbon dioxide at depth. This may affect the mycoflora by altering the pH of the surrounding area but it may also directly inhibit the growth of fungi. According to Burges (1958), who examined several fungi with regard to their growth response in conditions of reduced oxygen concentrations, there was little response, which suggests that the carbon dioxide concentration is the critical factor and not the lack of oxygen, as many species were affected by increasing the carbon dioxide concentration. Consequently even if spores are washed to the lower layers their growth may be inhibited by carbon dioxide (Burges and Fenton 1953). The concentrations of the two gases may vary over a short distance and with time depending on such things as the activity of the organisms and the diffusion of the gases to the atmosphere which will be affected by the soil type.

There is another factor which may influence the species of fungi which occur at depth. Sewell (1959) found that the isolates from the lower levels tended to be slow growing and often were not isolated from the upper layers. The upper layers of a soil are more favourable to fungal colonisation and therefore there are usually many isolates from a topsoil sample. Some of these fungi may be fast growing in comparison with those isolated at depth so that they may overrun the slower growing fungi which will not therefore be isolated in the upper layers. Consequently there is a qualitative difference which may be entirely artificial.

Table 3.5

Isolates/gram and total number of Species at different depths

	Topsoil	5cm	15cm	25cm
Site 1	100/67	58/43	46/44	21/23
Site 2	122/63	29/32	18/21	26/23
Site 3	138/48	53/35	46/36	53/32
Site 5*	902/83	914/75	741/80	689/73
	Added Topsoil	Former Topsoil	5cm to 15cm	15cm to 25cm
Site 4	289/59	181/73	112/74	136/15

*Only 10mg used in each sampling

In this study most of the sites show a decrease in the numbers of isolates and species with depth, however, they vary in the amount of decline. Site 4 is difficult to assess as topsoil was added which affected the layers physically and possibly by introducing organic material and inoculum. There is another problem in that the original

topsoil and 5cm layers were sampled throughout the two years. The topsoil was added in December 1970 and so was sampled eight times and the lowest level was only sampled prior to then. This means that the numbers are the result of different numbers of samples. However, the added topsoil still contains more isolates than the lower levels although the data is derived from four samples fewer, indicating that it is probably a more favourable habitat than the lower layers. The former topsoil and 5cm are similar in numbers of isolates and species so that they are probably similar environments and the change in depth may not yet have begun to affect the former 5cm level. Once the effect of the topsoil has declined then the effect of depth may appear.

At the other sites the various horizons were sampled throughout the two-year period. The relative lack of numbers in the topsoil at Site 1 is probably due to the fact that it is made up of mainly coarse material with many small stones which would not be a very suitable habitat for fungal growth, unlike Site 2 which is made up of fine material similar to the lower levels. The higher numbers in the topsoil may be due to the outside supply of inoculum in addition to that already present in the soil. The comparative richness of the lower levels of Site 1 is difficult to explain as they are from similar material as Site 2, possibly the difference in the physical structure of the topsoil means that the lower levels are not so shut off at Site 1 with regard to the exchange of gases with the atmosphere, and possibly the supply of some windborne inoculum penetrates the lower levels. These factors do not prevail at 25cm which drops to similar numbers at Site 2.

Site 3 has a similar pattern to Site 2 with the topsoil only differing from the other three levels although the numbers at depth

are much higher in Site 3 which may indicate the relative hospitality of the two sites. Site 5 does show some variation in the numbers of isolates/gram at different depths but the numbers of species are similar. This comparatively reduced variation is to be expected as the soil was regularly ploughed which disturbs the structure of the soil and reverses the levels. There are fewer isolates at the 15cm and 25cm levels probably due to the gradual accumulation of carbon dioxide as the soil lies fallow, since the soil is quite clayey which is poor for diffusion of gases. Also, during the fallow period there will be little in the way of nutrients reaching the lower depths after the residues have been utilised. It would seem that even at 25cm Site 5 is a much more favoured environment and a relatively well established one.

The number of species which occur at different depths generally shows a similar pattern as the number of isolates. In all the sites, with the exception of Site 4, there is a larger number of species isolated from the topsoil than the lower layers. This is also true for Site 5 where the numbers of species do not show much variation with depth probably due to the mixing of the levels by ploughing. In general the relatively high number of species in the topsoil may be due to the fact that the topsoil is a relatively favourable environment. It is also usually the first part of the soil which is colonised and will receive added inoculum from the air. Sites 1, 2 and 3 all show a marked decline with increasing depth. However, it is interesting that the topsoil of Site 3 contains fewer species than Sites 1 and 2 although there are more isolates/gram. It is not suitable for as wide a range of species to be found but those that do occur are able to grow comparatively well. There is not such a marked reduction in the number of species with increasing

depth at this site with all the lower levels containing a similar number. Site 1 shows a marked decline below the topsoil and 15cm which may indicate the relative hospitality of the different layers. Site 2 only shows a decline below the topsoil so that the two similar sites (1 and 2) do not show a similar pattern, which may be due to their physical differences. Site 4 is complicated by the addition of topsoil and the varied number of samplings at different depths. However, the former topsoil and the 5cm then 15cm contain a similar number of species. It would seem that the numbers of species are subject to the same conditions as the numbers of isolates so that their reaction is similar in each site.

Generally the isolates and species at depth are of infrequent occurrence and few in number. Site 5 is similar but the numbers of isolates is much higher. There is no evidence of the development of particular mycoflora at any given depth at any site. At Site 5 where the layers of soil are regularly mixed no particular mycoflora would have been developed, but at other sites it may be that they have not yet been established for a sufficiently long time to allow the development of a distinctive mycoflora.

In a closer examination of the spoil heap sites the numbers of isolates at different depths were compared between sites using regression analysis. Regression analysis assesses whether the numbers of isolates vary in the same way at two sites over the two-year period indicating whether there may be some common factors influencing the results at the different sites. The means may not be the same, but if they vary in the same degree then there will be correlation. Most of the combinations of depth and site showed no statistically significant relationship although visually some of them seemed similar. This emphasises that there are many differences

Table 3.6

Means of the number of isolates and correlation coefficients of comparisons of different depths between the Sites

SITE	HORIZONS	MEAN	COEFFICIENT (r)	P
1	25cm	4	0.81	0.001
2	25cm	5		
2	5cm	5	0.7	0.01
3	25cm	10		
1	15cm	9	0.75	0.01
2	25cm	5		
4	former Topsoil	35	0.66	0.01
2	Topsoil	24		

between the sites. Also it shows that quantitatively they do not follow a similar development or have the same factors acting in a similar manner. From all the possible combinations only four were statistically significant (Table 3.6). Two of these were between Sites 1 and 2 at the lower levels which are very similar in composition and so might be expected. The other two are between Site 2 and Site 3 and Site 2 and Site 4. As they are isolated occurrences there is little that can be deduced except that there is some quantitative similarity between these horizons.

It would seem that statistical analysis emphasises the differences between the sites. There is no indication of the relative colonisation of the sites to indicate their relative hospitality to a mycoflora. It may also be that there are many factors involved and the balance of these varies at each site.

These sites contain relatively few isolates when compared with other studies. It is more difficult to compare the numbers of species since this not only depends on the method of isolation,

but also on the number of isolations, which in this case was four plates from each level at each individual sampling, (Burges 1963). Also if a particular fungus is not isolated it does not automatically mean that it is not present in the soil merely that the method being used may not be conducive to its isolation. Throughout this discussion concerning the species, it must be remembered that those from Site 5 have been isolated from only 10mg of soil, and the number of species cannot be multiplied up like the isolates as there is not an infinite number of species.

In total 198 species were isolated during the two-year period, the majority of which were ephemeral occurring only once and in low numbers. The importance of these is difficult to assess for they may be beginning to colonise the soil or else they may not be easily isolated on soil plates or they may only be due to a stray spore which has been blown in and possibly washed down. The individual sites show some variation in the number of species isolated.

Table 3.7

The actual numbers of Species and as a percentage of total Species

Site 1	96	48%
Site 2	83	41%
Site 3	84	42%
Site 4	118	59%
Site 5	133	67%

As would be expected, the richer soil of Site 5 contains the most species. Site 4 also contains a relatively high number of species, probably due to the added topsoil introducing inoculum and a comparatively healthy environment. The other three sites contain

fewer isolates and it is interesting to note that Sites 2 and 3 are similar rather than Sites 1 and 2 which are from the same spoil heap. Site 3 generally contains more isolates/gram than the others, although this is not the case with the numbers of species. It would seem that in Site 3 only some species can survive, but those are relatively productive of isolates.

In considering the disposition of the species between the sites, 17% of species occurred on all sites (Table 3.8). This is not a reflection of their importance at each site as they may only occur once. However, most of the commonly occurring species are to be found in varying amounts at each site.

There is not a great deal of difference between the four spoil sites and the agricultural field, since only 12% of species are exclusive to Site 5 and most of these are occasional members of the soil flora as reflected by the soil plates. However many of the species on the spoil heaps are not widespread, in fact only two species occur on all four spoil sites but not on Site 5, and they are Emericellopsis species and Phoma violacea, neither of which occurs regularly during sampling. The reasons why Emericellopsis species should be found only in the spoil heap samples is not known. However, it is interesting to note that Phoma violacea has been found to grow on lead paint surfaces (Eveleigh 1961) and this might explain its occurrence in these soils. Further, since it has few fungal competitors here the chance of its being isolated is correspondingly increased. The chance of the same species occurring at four sites only is not very high so this may account for there only being two species.

Of the total of 198 species 66 of these, that is 33%, are isolated from one site only during the study. All of these species occur only once and usually only in a single isolation so that their

Table 3.8

List of Species common to all Sites

Absidia coerulea

Alternaria humicola

Aspergillus chevalieri
var. intermedius

Aspergillus fumigatus

Aureobasidium pullulans

Botrytis cinerea

Candida species

Cephalosporium acremonium

Cephalosporium curtipes

Cladosporium herbarum

Coniothyrium fuckelii

Fusarium sambucinum
var. caeruleum

Fusarium solani

Mucor hiemalis

Oospora sulphurea

Penicillium brevi-compactum

P. citrinum

Penicillium cyclopium

P. frequentans

P. funiculosum

P. janthinellum

P. jenseni

P. lilacinum

P. meleagrinum

P. notatum

P. ochraceum

P. ochro-chloron

P. spinulosum

P. simplicissimum

Phoma glomerata

Phoma herbarum

Sterile Mycelia

Trichoderma viride

Yeast species

ecological role is doubtful.

The spoil heap sites have been compared with Site 5. Site 4 and 5 contain 85% of all the species which is to be expected, since they are the richest sites in organic matter. Of the 85% the sites have 49% in common, indicating that they are quite similar qualitatively. This is probably a reflection of the influence the addition of top-soil had on the flora at Site 4, although this cannot be categorically stated since there is nothing really to compare it with. The species in common are quite varied including several Phycomycetes and Penicillia.

However, when the other three sites are compared with Site 5 the numbers of species isolated are quite high. This is probably due to the large number which occur at Site 5. In a comparison of Sites 1 and 5, 79% of all the species were isolated from these sites and of these they had 45% in common. In comparison of Sites 2 with 5 and 3 with 5 there were 77% of the total species isolated in each combination. Sites 2 and 5 had 51% of these in common while Sites 3 and 5 had 41% in common. It would seem that all the sites are relatively similar to Site 5. This is to be expected, since the likely colonisers and stray isolates occurring in the spoil will be normal soil inhabitants of which a large number occur in the more suitable Site 5.

Some comparisons were conducted between the spoil sites. It was thought that Sites 1 and 2 might be similar as they are from the same spoil heap. Of all the fungi isolated 58% occur on these spoil heaps and 52% of these are common to both sites. Although the fungal flora is not rich the number shared is quite high, being slightly more than half. This indicates that the sites are quite similar qualitatively although Site 2 is poorer in total number of species.

The species in common are varied in type. Site 1 was also compared with Site 3 since the latter was thought to be more hospitable in a comparison of the isolates/gram. Only 25% of all the species isolated occurred on these sites indicating that they contribute very little to the whole. Of these, 38% were shared between the two sites, indicating that they have little in common although there is some slight similarity.

Site 3 was compared with Site 4 since both of them had received an application of topsoil, although at different times and it was thought that there might be some similarity of the species as a result. Here 72% of the total species were isolated from these sites of which Site 4 contributed the greater number and 39% of these were shared by the two sites. So there is some similarity between the sites and it may be that Site 4 may settle down and become like Site 3 qualitatively.

In summation there are some similarities between the sites but these are not very marked and the importance of the total comparisons is dubious since this only registers occurrence and not the relative importance of the species in the various sites.

There is a wide range of species occurring in these soils, with only a few of them occurring regularly. Several Phycomycetes were isolated during the investigation, the majority in Sites 4 and 5. They have been found in significant numbers in alkaline soils at Lakenheath Warren (Warcup 1951) so that the pH is not an important prohibitive factor. They consist mainly of Absidia species, particularly Absidia coerulea and Absidia spinosa, and Mucor species, especially Mucor hiemalis, M. microsporus and M. varians. However, these two genera are found in samples from each site, although they occur only occasionally in Sites 1, 2 and 3. In

addition some Zygorhynchus and Rhizopus species are found in Sites 4 and 5. The marked occurrence of these species in Site 4 and 5 is probably due to the presence of organic matter unlike the other sites which have very little. These fungi depend mainly on relatively simple compounds, e.g. sugars and so are usually to be found where there is a supply of fresh material which means in association with surface vegetation (Garrett 1951). This also explains their preference for the topsoil layer as sugars would not reach the lower levels, but be utilised near the surface. The sporadic occurrence of these fungi in the soil at other sites may be due to stray spores blown in or some organic debris. However, since they are rapid growers if there is some material which the fungus can utilise then there may be a large number of isolates, as occurred in the topsoil of Site 3. This may be associated with the scattered vegetation at this site.

There are some Ascomycetes present in these soils; there may be more as no particular method, such as soil steaming, was employed to isolate them (Warcup 1951). Chaetomium species were the main ascomycetous species and they occurred only occasionally in the topsoil and upper layers of all sites with the exception of Site 3. However, in Site 5 they occurred in all the layers which possibly reflects the comparative richness of this soil and its mixing. Ascomycetes are generally found in association with animal dung which was not observed during sampling, so that it may be as a cellulase producer that the Chaetomium species occur (Gray and Williams 1971). Cellulose would be expected to occur mainly in Site 5 and that is where most of the isolates are found.

As with most soil studies the majority of species isolated belong to the Fungi Imperfecti. This may in part be due to the

use of the soil plate method which many of them favour, but even so they are thought to be important members of the general soil flora. The major genera, either with regard to the number of species or to number of isolates, are Aspergillus species, Fusarium species, Gliocladium species, Penicillium species, Phoma species, Trichoderma species, most of which are considered to be normal regular inhabitants in soils (Waksman 1944).

The major genus, containing the most species is Penicillium as in some other studies (Brown 1958, Nicholls 1956), however, this finding may have been influenced by the use of the soil plates which favour heavily sporing species such as these. Many of the species are isolated incidents and so are possibly not major contributors to the mycoflora of these soils. Some, however, such as Penicillium canescens, P. cyclopium, P. decumbens, P. frequentans, P. funiculosum and P. lilacinum occur quite regularly and are probably important members of the mycoflora. Most occurred regularly in samples from Site 5, and Warcup (1957) also isolated several Penicillium species in his study of a wheat field. Since Site 5 is richer in organic matter than the other sites and also contains less lead, it is not surprising that it should contribute the largest number from a probably well established mycoflora. Site 4 also contains markedly more than the other three sites, the species P. lilacinum, P. luteum, P. ochro-chloron and P. simplicissimum, occurring in greater number than the other Penicillia at Site 4. All of these species are considered normal soil inhabitants and occur mainly in the topsoil samples with the exception of P. lilacinum which occurs mainly in the spoil material at the lower levels. This fungus and P. ochro-chloron are tolerant of many chemicals (Raper and Thom 1949) which may explain their presence here. However, it is P. ochro-chloron which

is metal tolerant (particularly copper) but it is P. lilacinum which occurs mainly in the spoil material. It may be that the environment is not conducive to P. ochro-chloron in other ways. P. lilacinum occurs in alkaline soils in quite large numbers (Nicholls 1956), and it is obviously able to exist at depth and to tolerate the conditions there.

The other three sites show little predominance of any one species and although the regular ones generally occur in greater numbers they are not so dominant. The Penicillia as a whole occur at all depths, with those which occur more regularly usually being found equally distributed in every layer. The regular occurrence of these at depth indicates that they may grow there since according to Burges (1950) the spores of Penicillia do not move easily through the soil and movement may account for only occasional isolations.

There are several species of Aspergillus present in these soils but they only occur in a few samples and not in very large numbers with the exception of Aspergillus fumigatus. This fungus seems to be an important member of the mycoflora of each site, occurring regularly at all sites except Site 5. At Site 4 it was originally present in large numbers; these fell dramatically after the addition of topsoil, but were gradually building up again towards the end of the observational period. It is found in most soils and can tolerate the harsh and alkaline conditions of poor muds of salt marshes (Pugh 1962). It is also the type of fungus well suited to the soil plate method of isolation which may have enhanced its importance. Its numbers do fluctuate throughout the sampling period but they show no regular pattern. It is usually found at all levels in the soils and only in Sites 1 and 2 does it show a preference for the upper layers which may reflect the relative paucity of the

two sites.

Fusarium species contribute quite a lot to the mycoflora of Site 5 which is in keeping with the idea that they are normally associated with cultivated soils (Bisby et al. 1935) and often in a commercially important scale as they can attack some crops. However, they are found in the other sites to varying extents, Sites1, 2 and 3 contribute very few and there are some more in Site 4 probably due to the topsoil which was added.

Fusarium sambucinum v. caeruleum is the main species found in Site 3 and it is restricted entirely to the topsoil fraction. This may be a reflection of the fact that topsoil was added to this site at some time and there is still some scattered vegetation. In Site 4 most of isolations of Fusaria occur in the added topsoil and when found in other horizons it was usually after the addition of topsoil, F. sambucinum v. caeruleum is the main isolate. In Site 5 both F. culmorum and F. sambucinum v. caeruleum occur regularly and in large numbers. F. culmorum is more often isolated due to the influence of the Barley crop as it is often found in association with it.

Gliocladium roseum is the only member of this genus which occurs in any numbers during this investigation. It occurs mainly in Site 4 and Site 5 with only an occasional isolate from Site 1. In Site 4 it is a regular member of the mycoflora occurring mainly in the added topsoil but also in the former topsoil in considerable numbers. It is also regularly isolated in the samples from Site 5 but is found in greater numbers at the lower three levels. The reason for this preference is difficult to suggest since these layers are regularly mixed and there is probably an adequate supply of nutrients for the fungus. It occurs very infrequently at the other three sites. It is usually considered a true soil fungus (Bisby

et al. 1935) as it occurred widely in soils in Manitoba, however, it is usually associated with fairly favourable soils which the spoil heaps are not. Nicholls (1956) did not find it regularly in her examination of chalk soils nor Warcup (1951) in his study at Lakenheath Warren so the pH may also be influencing it as well as the lack of nutrients.

Phoma species are not generally considered to make an important contribution to the mycoflora although they do occur in soils. In this investigation several species were isolated but Phoma herbarum was the main one. It was a regular and numerically large member of the mycoflora of the four spoil sites. It did not occur in any important amount at Site 5 and it may be that the increased competition associated with the richer supply of nutrients affects its appearance in the soil samples.

In Sites 1, 2 and 3 Phoma herbarum mainly occurs in the topsoil particularly in Site 3. It may not be able to tolerate the effects of depth very well or it may be that there is very little in the way of nutrients and so it occurs only where there might be some. In Site 4 it rarely occurs in the added topsoil which may be due to the same reasons it does not occur in the samples of Site 5. This fungus is able to tolerate the harsh conditions of the spoil heaps with their poor supply of nutrients and the lead content. It is usually found associated with plant material, but strains of Phoma herbarum have been shown to be able to attack lead paint surfaces (Boerema 1964) and this may be the influential factor allowing the fungus to occur in such large numbers on the spoil heaps.

Trichoderma viride is another species commonly found in soils, Jensen (1931) found it mainly in acid and waterlogged soils, but it

is generally considered to occur in many soils. Warcup (1951) found it in all his soils including the extremely acid and alkaline ones. It is most important in Sites 4 and 5 predominating in the upper layers and particularly the added topsoil of Site 4. It also occurs mainly in the topsoils of Sites 1 and 2 regularly but in low numbers and it hardly occurs in the samples from Site 3. It is considered to prefer good soils which is why it occurs in larger numbers in the richer sites of 4 and 5 probably associated with the vegetation.

Qualitatively in this study it was decided to compare the regularity with which some of the fungi isolated occurred in the samples. It was thought that this would indicate the important fungi composing the mycoflora of the sites and show any similarities on a slightly more reliable basis than just occurrence. Sewell (1959) also suggested that it counteracted the high sporing capacity of some fungi which suits them to the soil plate method. At each site there are several fungi which were isolated in all the four seasons sampled. Site 5 contained more than the spoil sites reflecting in all probability Waksman's idea (1944) that the more fertile soils usually contain the greater number of isolates and species. This may indicate the comparative stability and richness in comparison with the spoil. Site 3, despite containing quite a large number of isolates has the fewest species occurring regularly.

At Sites 1 and 2 the regularly occurring species are similar in spite of gross differences between the sites. This is probably influenced by their chemical similarity in constitution, although they are not identical physically. This may indicate that they are in a similar state of colonisation. One species - Aspergillus versicolor - occurs in Site 1 regularly but not in Site 2. Since it was only isolated in four samples it is only just occurring in

Table 3.9

Species which occur in the four seasons sampled

SITE 1

Aspergillus fumigatus
Aspergillus versicolor
Coniothyrium fuckelii
Epicoccum purpurascens
Penicillium frequentans
Penicillium funiculosum
Phoma herbarum
Trichoderma viride
Sterile Mycelia

SITE 2

Aspergillus fumigatus
Coniothyrium fuckelii
Epicoccum purpurascens
Penicillium cyclopium
Penicillium frequentans
Penicillium funiculosum
Phoma herbarum
Trichoderma viride
Sterile Mycelia

SITE 3

Aspergillus fumigatus
Coniothyrium fuckelii
Penicillium cyclopium
Phoma herbarum
Sterile Mycelia

SITE 4

Absidia spinosa
Aspergillus fumigatus
Coniothyrium fuckelii
Cylindrocarpon olidum
Gliocladium roseum
Mucor varians
Penicillium funiculosum
Phoma herbarum
Trichoderma viride
Sterile Mycelia

SITE 5

Absidia spinosa
Coniothyrium fuckelii
Fusarium culmorum
Fusarium sambucinum var. caeruleum
Gliocladium roseum
Mucor microsporus
Penicillium canescens
Penicillium cyclopium
Penicillium decumbens
Penicillium frequentans
Trichoderma viride
Verticillium candelabrum
Sterile Mycelia

sufficient number and conditions may not be so suitable in Site 2. At Site 2 there is a Penicillium - P. cyclopium - which occurs regularly at this Site but not at Site 1. As this fungus produces many spores and is found in air samples it may be that it occurs to some extent as an airborne contaminant of the topsoil. The topsoil at Site 2 is more suited to colonisation than Site 1 which is very coarse and contained fewer isolates during sampling than that at Site 2. Epicoccum purpurascens occurs regularly only in these two sites. It is usually a common secondary invader on plants but is capable of growing in the soil. There are only occasional plants here so that it must be some conditions occurring at these sites making them suitable. At Site 3 there are only five species which occur regularly during the two-year period. These all occur at one or more of the other sites so that there appears to be nothing distinctly different about this site, except that there are slightly fewer fungi occurring regularly. An interesting absentee from this site is Trichoderma viride which was regularly found at all the other sites. Obviously there is something inhibiting the development of this fungus at this site since it can normally compete well on the soil plate so it probably does not occur in this soil to any marked extent.

Site 4 which contains the largest number of regularly-occurring isolates amongst the spoil heap samples may have been affected by the addition of topsoil. Consequently some species are similar to the other spoil sites and some are similar to Site 5. There are two Phycomycetes occurring regularly here while none occur in the other spoil sites. Since these are usually dependent on simple nutrients their occurrance is probably

associated with the surface vegetation which is found here. One of the Phycomycetes - Mucor varians - occurs regularly at this site but the fact that it is a Mucor species is probably the relevant part. One other fungus occurs regularly at this site and no other and that is Cylindrocarpon olidum.

Site 5 contains the largest number of regularly occurring species due to its being a more suitable and normal environment. Six of the species are unique in occurring regularly at this site. Two of these belong to the genus Fusarium and their occurrence is probably closely associated with the growth of a barley crop. Two others are Penicillia which are normally found growing strongly in soils and are also suited to isolation on soil plates. The other two are Mucor microsporus and Verticillium candelabrum. The importance of the former is probably as a Phycomycete associated with the vegetation of the site, while the latter is suited by the conditions here.

Coniothyrium fuckelii is the only species which occurs regularly at each site. Sterile mycelia also occur regularly but they form a large part of most soil more due to an inability to cause them to fruit rather than them all being sterile. Coniothyrium fuckelii is not normally considered to be an important member of the soil mycoflora. It may be that it is not isolated readily from normal soils or soil plates or that in fact it does not compete well in the soil and so is not found very often. In these soils where there are fewer isolates it is able to survive albeit not in great numbers in the soil and on the soil plates. It may also be that this fungus can tolerate lead in the soil and so that it can take advantage of the reduced competition.

Two fungi are isolated regularly only from the four spoil sites. These are Aspergillus fumigatus and Phoma herbarum. Both

of these are normal soil inhabitants. However, Phoma herbarum is not normally an important member of the soil mycoflora while in these soils it is not only a regularly occurring fungus but it also contributes a large number of isolates. It may be that the strong growth of this fungus is due to the reduced competition but also may be associated with the lead content and relative paucity of these soils. Although A. fumigatus does not occur in every season from Site 5 it does occur in seven samples and in fairly large numbers. It is likely that it was an adverse alteration in the micro-environment at Site 5 rather than a distinct difference from the other sites which prevented its occurrence at all times.

In addition to the examination of the regularly occurring species, which does not take into account the numbers in which they occur and so does not indicate their inter-relationships, it was also decided to examine the numbers contributed by the various species to the whole (Thornton 1956). Thornton suggested that the degree of variation may indicate the closeness of two soils. These fungi will be affected by their ability to survive and compete on the soil plate unlike Thornton's findings which were from immersion plates. All of the species which contributed more than 5% of the total numbers were regularly occurring; this is understandable as they have time to amass the numbers required unlike those which are not regularly isolated.

There is some variation between the sites. Sites 1 and 2 are very similar, the exception being the presence of Coniothyrium fuckelii at Site 2 in large numbers, however, it does occur regularly at Site 1, but not in sufficient number. This fungus is also a major contributor to the bulk in Site 4, it was also found in considerable numbers by Brown (1958) in both acid and alkaline dunes but mainly the latter. Some isolates have been known to tolerate high concentrations

Table 3.10

Species each contributing 5% or more of the isolations

Site 1

Aspergillus fumigatus

Phoma herbarum

Trichoderma viride

Sterile Mycelia

Site 2

Aspergillus fumigatus

Coniothyrium fuckelii

Phoma herbarum

Trichoderma viride

Sterile Mycelia

Site 3

Aspergillus fumigatus

Phoma herbarum

Sterile Mycelia

Site 4

Coniothyrium fuckelii

Gliocladium roseum

Phoma herbarum

Trichoderma viride

Sterile Mycelia

Site 5

Aspergillus fumigatus

Gliocladium roseum

Trichoderma viride

Sterile Mycelia

of copper sulphate (King and Keplinger 1951) and it may perhaps be able to tolerate lead to some extent and so occur here. It is interesting that although this fungus is isolated regularly from each site, it is only in large numbers from two of the sites. It can obviously grow in these soils but the amount varies with some perhaps being more suitable than others. Aspergillus fumigatus occurs in all lists except that for Site 4 where it declined after the addition of topsoil. Also despite not occurring regularly at Site 5 it does manage to contribute more than 5% indicating that when present it is a strong grower. With the exception of Site 3

which is very restricted in the number of species which contribute large numbers, there are some other species which occur in the other sites. Trichoderma viride is important in the other sites; it is a normal soil inhabitant but its relative importance may be relevant to lead contaminated and poor soils. Phoma herbarum occurs on all the 5%+ spoil heap lists as it did when considering its regularity, so that it is probably an important member of the mycoflora of these soils. Gliocladium roseum is a major contributor to Sites 4 and 5 where it occurs regularly, it may indicate the gradual increase of organic matter in the soils as it is able to tolerate some lead contamination.

It is interesting that Site 3 which is quantitatively relatively rich is lacking in total numbers of species and in numbers which occur in significant amounts. It would seem to be relatively favourable but only to very few fungi and it does not really have a stable mycoflora. This site may be more variable than the others in its material as it consists of the mine waste.

It would seem, therefore, that there is little to indicate a distinct flora qualitatively since most of the isolates do occur in other soils, however, their numbers are slightly different with Phoma herbarum and Coniothyrium fuckelii being more important than normally. It is perhaps significant that there are few fungi which occur in appreciable amounts or with regularity which is indicative of the inhospitality of the sites including even the agricultural field.

These fungi are those which are able to tolerate the conditions occurring in the spoil heaps. One of the main factors in this environment is the lead content of the soils. These fungi which are main contributors to the mycoflora would seem to be able to adapt to or tolerate the presence of lead. Some grasses, e.g.

Agrostis tenuis (Bradshaw 1952) have been shown to adapt to the presence of toxic metals although they are not normally tolerant. It was thought that fungi may follow a similar pattern. An experiment was conducted comparing the growth of two isolates from different sources of some species (see Chapter 5, Section 5.11). The experiment showed that some fungi are naturally tolerant of lead, e.g. Trichoderma viride. This means that these fungi, having infected an environment containing lead would be able to grow if the conditions were suitable for development. Other fungi in the experiment had developed a tolerance to lead while existing on the spoil or else only some strains of a species are tolerant of lead and these developed on the spoil, e.g. Phoma herbarum. This may be a partial explanation of the presence of some of the major species in these sites, but there are also other factors involved in allowing a fungus to develop on a particular substrate.

In summation the numbers of isolates from these five soils are very low when compared with normal soils. Also there are only a few species which are able to tolerate conditions here and grow to any marked extent. There seem to be two possible reasons for the low numbers, firstly the paucity of the spoil material is probably not conducive to reasonable fungal development. However, this factor probably does not apply to Site 5 (the agricultural field) as it would seem to be a relatively favourable environment. The second factor, lead contamination, may be the main influence on Site 5 butit is probably active at all sites. However its effect does not seem to be direct or immediate, but its influence possibly gives rise to a general lowering of numbers. Both pH and soil moisture do not show any direct effect on the mycoflora but they are probably active. Qualitatively there does not seem to have

been any development of a distinctive mycoflora as the fungi occurring here are cosmopolitan. The agricultural field is similar to normal soils with smaller numbers than usually occur. However, there are two fungi which seem more important than in normal soils - Coniothyrium fuckelii and Phoma herbarum. The former is a regular member of all the soils though not in large numbers. The latter is important only in the spoil heap soils.

It may be that there has not been sufficient time to allow for the development of a specific mycoflora adapted to the particular conditions of the spoil.

SECTION II

EXPERIMENTAL STUDIES

INTRODUCTION

The toxic effect of metals has long been recognised, but it is only in this century that it has been closely studied (Hammond 1969). Passow, Rothstein and Clarkson (1961) have reviewed much of the work on the effect of heavy metals mainly on animal tissue. However, some metals are necessary in very small quantities to the metabolism of many organisms including fungi. There have been some reviews concerned with metals and fungi from a nutritional viewpoint (Foster 1939 and 1949, Perlman 1949), and also their toxic effects on fungi (Horsfall 1956). Steinberg conducted much of the pioneer work on mineral requirements of fungi and he reviewed the subject (1956) and concluded that since they are required in such small quantities their role in the metabolism of the fungus must be catalytic. There have been more detailed studies concerning the activity of the metals in fungal metabolism. Chesters and Rolinson (1951) examining the effect of zinc on carbohydrate metabolism found that not only zinc but also deficiencies in other nutrients affected the efficiency of glucose utilisation by the fungus Aspergillus niger. Takagi (1957) examined the effect of copper and halogen ions on the colour of Aspergillus conidia and showed that the mutants producing yellow conidia due to a deficiency of copper uptake could produce green conidia if halogen ions were supplied with the copper indicating that the effects of metals is not simple.

The fact that increased amounts of metals are poisonous has been utilised in the manufacture of fungicides used in the protection of commercial plants, seeds and various material which may undergo decay. McCallan and Wilcoxon (1934) screened many elements for their

fungicidal ability and their relation in the periodic table. Several elements were toxic and they suggested some including lead which may be of possible use as fungicides. One of the most important elements used for its fungicidal ability is copper which is part of one of the oldest and most successful fungicides - Bordeaux Mixture. Martin (1946) summarises the effect of copper and various other fungicides on the fungus and the plant.

Horsfall (1956) summarised the action of metals on fungi and found that much of the evidence suggested that the surface of the spore was an important area of activity but that some metal did penetrate the cell and once inside the cell formed chelates with the amino acids and proteins. Rothstein and co-workers studied the effect of uranium on cell surface of yeast, and suggested that the uranium complexes with groups on the cell surface and then possibly penetrates into the cell slowly (Rothstein and Larrabee 1948). It would seem that some of these groups are active in anaerobic glucose consumption since it is inhibited by uranyl nitrate (Rothstein, Frenkel and Larrabee 1948). Later studies showed that uranium acts specifically on hexoses, affecting active transport by the membrane (Rothstein, Meier and Hurowitz 1951).

Somers (1963) studied copper in relation to the spores of Alternaria tenuis, Neurospora crassa and Penicillium italicum. He suggests that in N. crassa the initial uptake is generally an ion exchange reaction followed by a permeation process. He also shows that the cell walls of A. tenuis and P. italicum have a greater affinity for copper than was previously supposed. Since high levels of copper were necessary to produce a fungicidal reaction in N. crassa and A. tenuis Somers suggests that the reaction is unspecific or else the fungi are able to detoxify the metal in some way.

Some work has been done with lead and Aspergillus niger (Zlochevskaya and Rabotnova 1966, 1968 and Zlochevskaya and Rukhadze 1968). Initially Zlochevskaya and Rabotnova (1966) experimented to find a medium suitable for experiments with lead and then to train the fungus to grow well on the medium. They found that there was a reduction in biomass but not spore production when lead was present as free ions at a concentration of 269mg/litre in the medium. However, growth was eventually prevented in a medium where lead was present in a complex with cysteine hydrochloride at 97.2mg/l. Zlochevskaya and Rabotnova (1968) studied the effect of lead complexes on the growth of Aspergillus niger and showed that there was variation in the toxicity of the chelates but that none were more toxic initially than the lead ion. The toxicity of the compounds decreased with growth of the fungus. They concluded that the action of lead was 'bacteriostatic' and not 'bactericidal'. Zlochevskaya and Rukhadze (1968) examined the effect of some other lead compounds on the growth of Aspergillus niger and found that lead ions are toxic when in readily dissociating lead salts or in lipoid soluble complexes. They do not explain how lead produces the effects they observed.

With increasing industrialisation many metals have been released into the environment in greater concentrations than previously. Mining has given rise to areas of waste material containing large amounts of heavy metals and it has been shown that some grasses particularly Agrostis tenuis are able to tolerate the metals and so colonise these areas (Bradshaw 1952). As a result this grass has been studied to examine its ability to tolerate these high levels of poisonous substances, particularly zinc and copper (Turner 1967, 1970 and Peterson 1969). It seems that the cell wall (particularly the pectate fraction) acts as the major accumulator of the metals

(Peterson 1969). However, other cell components apart from the cell wall can also accumulate lead to some extent. More lead can be accumulated by the tolerant plants than the nontolerant plants (Turner and Marshall 1971). Reilly (1969) suggested that the high amount of copper held by the leaves of Becium homblei plants from copper-containing soils was complexed with the structural components, probably proteins, since it could not be extracted by the solvents used.

Other plants, e.g. Cladonia rangiformis (Brown and Slingsby 1972) have also been found on metal rich sites or able to tolerate high amounts of metals such as mosses. Generally it is thought that mosses absorb the metal from the air and they can act as indicators of pollution (Ruhling and Tyler 1968, 1969). Hyalocomium splendens is able to sorb copper and lead from dilute solutions and it is thought that these ions are sorbed from rain or snow by the moss carpet. The ions are also found in conjunction with the cell wall as in the previously mentioned plants (Ruhling and Tyler 1970).

The present ecological studies were to see whether fungi are able to tolerate heavy metals particularly lead. This section is to examine the effect of lead on the growth of fungi more closely and to see if there is any adaptation or selection towards lead tolerance by those fungi living in lead contaminated soils. There have been some studies on the adaptation of fungi to metal toxicants, some of which were reviewed by Ashida (1965). Parry and Wood (1958) studied the adaptation of Botrytis cinerea to copper and mercury salts. They produced strains which were resistant to higher concentrations of copper sulphate and phenyl mercuric acetate by gradually increasing the concentration to which the mycelium was exposed. To enable these strains to grow the inoculum had to be placed at the side of the

liquid medium where it was perhaps in contact with less copper. The process of adaptation was not a simple one and they suggest that it is unlikely that the strains would be important in the field. This is reflected by the fact that fungi do not seem to have been as able as bacteria to produce resistant strains. Although B. cinerea could tolerate a higher initial concentration when grown on solid media no increase in resistance was shown. Partridge and Rich (1962) using Penicillium notatum, Sclerotinia fructicola and Stemphylium sarcinaeforme found that prolonged exposure to various fungicides not only gave rise to increasing growth and sporulation, which in the end compared favourably with the control, but also to growth occurring at progressively higher concentrations. They found that the changes in tolerance were 'semipermanent' and may reflect adaptations of the metabolism to avoid the enzyme system blocked by the fungicide.

In the present work on the effect of lead on the growth of fungi Aspergillus fumigatus was used. This fungus was selected since it was isolated in large numbers in the preliminary investigation of the area to be studied and in time proved to be quite important in the mycoflora of the soils. Other experiments with additional species of fungi were conducted to see if there was any variation in the tolerance of lead between isolates of a given species and between species.

CHAPTER 4

THE EFFECT OF LEAD NITRATE ON THE GROWTH OF ASPERGILLUS FUMIGATUS FRES. ON AGAR MEDIUM

In the previous section results of an ecological investigation carried out on lead contaminated soils have been examined and discussed. In addition to the ecological studies it was decided to conduct some experiments to ascertain the effect of lead on some microfungi and one species in particular, Aspergillus fumigatus Fres. The initial experiments were carried out on solid media.

4.1 Effect of some low concentrations of lead nitrate on the growth of Aspergillus fumigatus Fres.

This experiment was conducted to examine the effect of a range of concentrations of lead nitrate on the pattern and the amount of growth of Aspergillus fumigatus Fres.

4.1.1 Experimental Procedure

The technique used was outlined in Chapter 1. Potato Dextrose agar was made up in test tubes containing 15ml agar, which is sufficient for one plate. The agar was autoclaved and allowed to cool, when cool one millilitre of the appropriate lead nitrate solution was added to each test tube. The test tubes were then shaken to try to ensure thorough mixing. The plates were then poured and allowed to solidify. The concentrations of lead used in this experiment were (in p.p.m.):- 6.25, 12.5, 25, 50, 75, 100, 200, 300, 400 and five plates were made up at each concentration. The plates were inoculated from cultures, which had been growing on agar slopes in medical flats for 7 - 21 days, using a tungsten inoculating needle. The inoculated

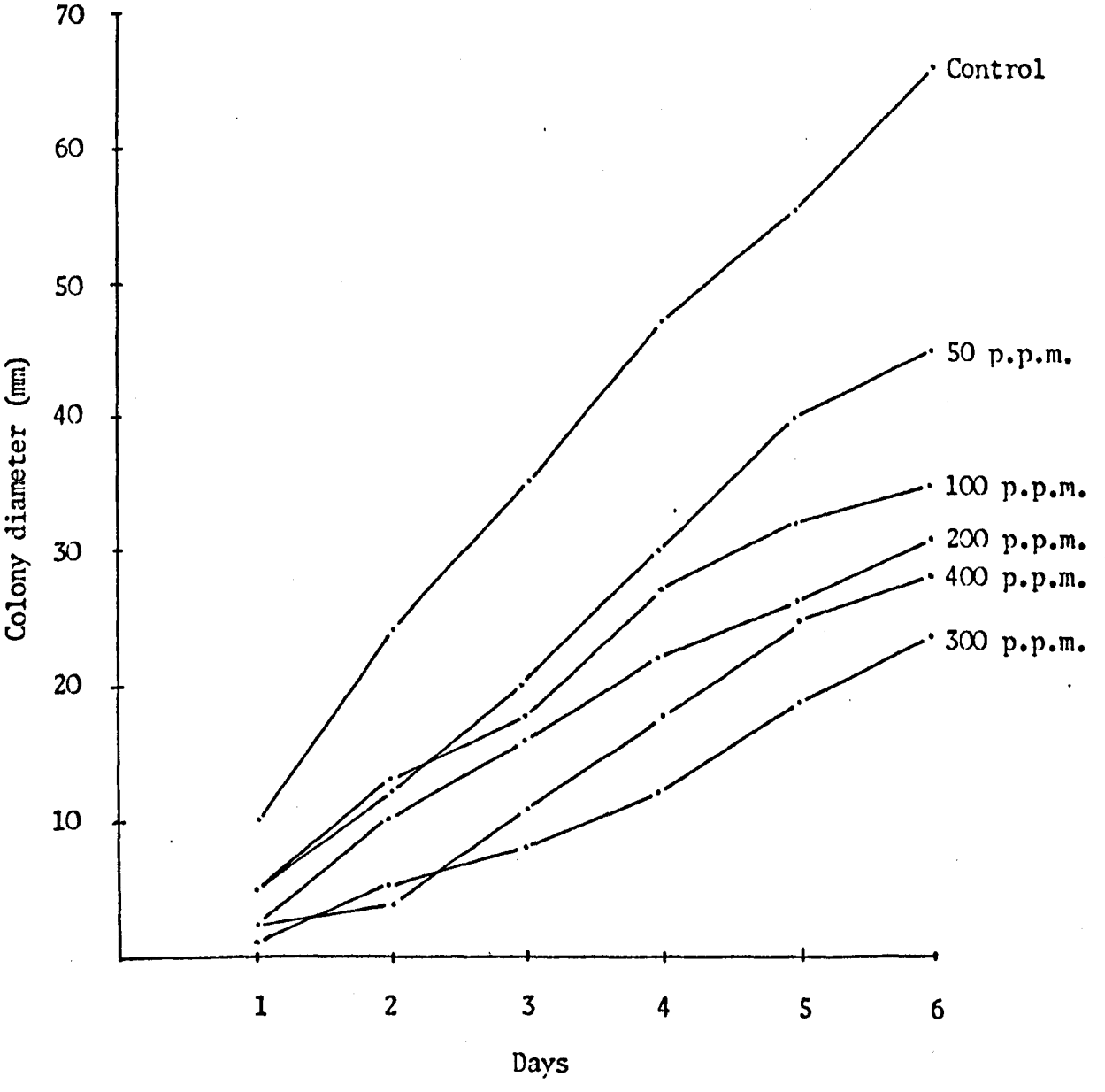


Figure 4.1

Growth of A. fumigatus in a range of lead concentrations on agar medium

plates were placed in a constant temperature room at 25°C and the colony diameter was measured daily.

4.1.2 Results

The results are expressed as the average diameter of the colony in millimetres and some are presented graphically (Fig. 4.1) and Table 77. The general trend is that growth as measured by increasing colony diameter, decreases as the concentration of lead increases. In general the control shows greater growth than the colonies on the media containing lead nitrate, the growth pattern being one of regular increase which varies in its amount between the different concentrations. Up to a concentration of 25 p.p.m. there is little difference in the amount of growth at the different concentrations, the growth is slightly diminished at 50 and 75 p.p.m. At 100 p.p.m. and the higher concentrations the effect of the addition of lead becomes more marked. At these concentrations growth is not only less but is somewhat slower to begin. Up to day 4 all the concentrations showed a similar amount of growth except 12.5 and 25 p.p.m. which had grown more and 300 and 400 p.p.m. which had grown less. After day 4 growth begins to slow down at 50 p.p.m. and higher concentrations.

4.2 The effect of a large range of concentrations of lead on *Aspergillus fumigatus* Fres.

It was decided after the previous results to test a range of high concentrations of lead on the growth of *Aspergillus fumigatus* Fres. and to try and ascertain the concentration which prevents growth.

4.2.1 Experimental Procedure

The technique which was used was outlined in Chapter 1. The agar was made up in 75 ml aliquots owing to the large numbers involved

in the experiment. This is sufficient for five plates. The appropriate concentration of lead was added to each flask of agar and mixed as thoroughly as possible. The strength of the lead nitrate solutions added were such that the agar contained the following lead concentrations (in p.p.m.):- 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, five plates were poured at each concentration.

Each plate was inoculated with a 3mm disc of agar cut from the edges of a colony (Baghdadi 1970). The diameter was measured daily.

4.2.2 Results

The results are expressed as the average diameter of a colony in millimetres. A graph has been drawn to show the pattern of growth at various concentrations (Fig. 4.2). Table 78 shows the diameters of the colonies at all the concentrations at which there was growth.

After 8 days growth on the control and 100 p.p.m. had almost reached the edge of the plate. As the amount of lead in the agar increases so the amount of growth decreases until there is no growth about 1600 p.p.m., since at the higher concentrations growth did not always occur on all five plates. The average amount of growth may vary quite considerably. There is a delay in the onset of growth and growth becomes slower as the amount of lead increases. This delay becomes more marked at 1000 p.p.m. and above until at 1600 p.p.m. there is no initiation of growth in the time period studied.

At the lower concentrations there is a distinct threshold below which there is a reasonable amount of growth and above which there is a marked decrease in the amount of growth. This difference occurs as in the previous experiment between 200 p.p.m. and 300 p.p.m. Above 400 p.p.m. growth is very limited and is more gradual until the later days.

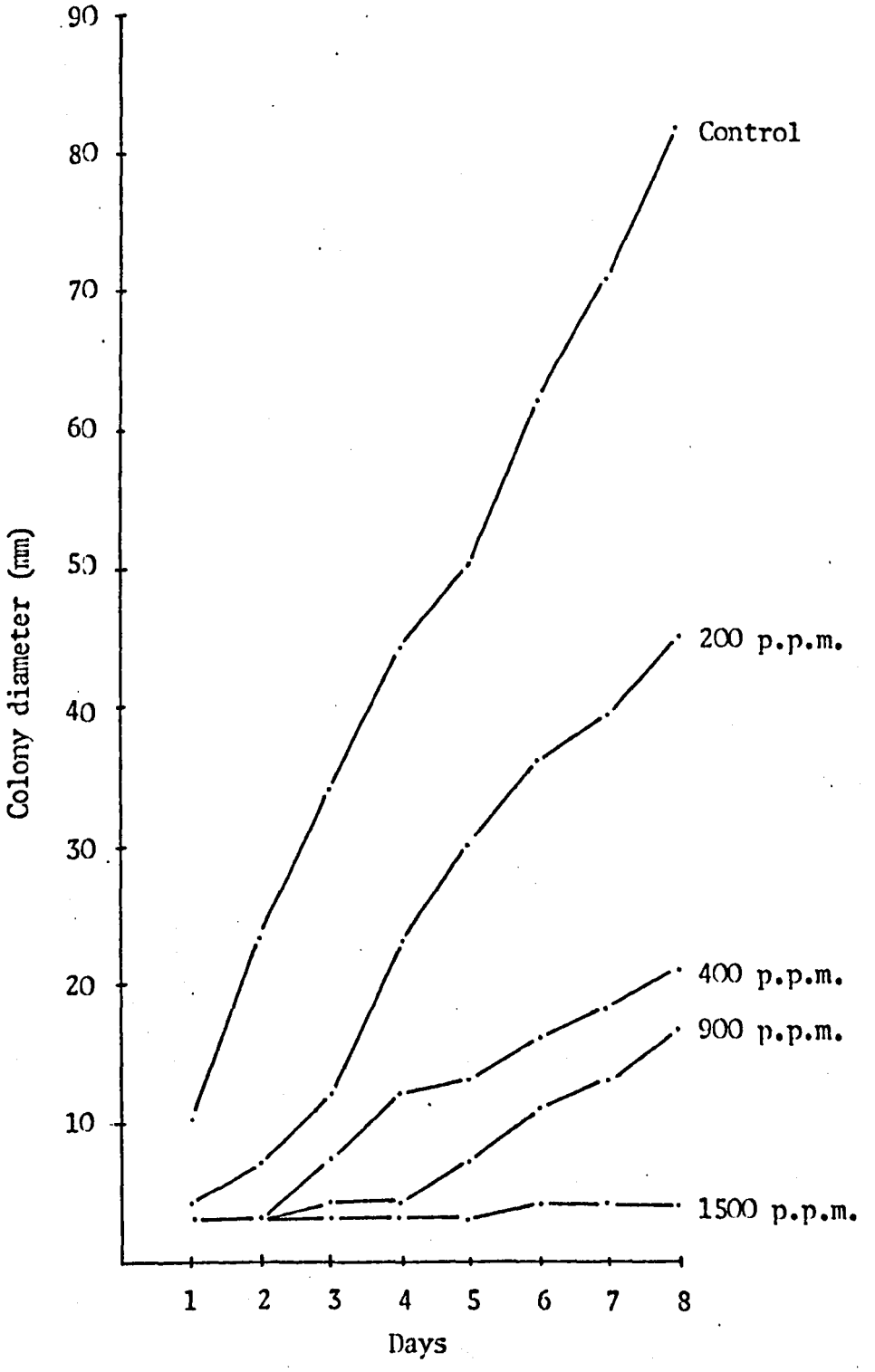


Figure 4.2

Growth of A. fumigatus in a range of lead concentrations on agar medium

The main feature of the results is that growth continues to decrease as the lead content increases, and the initiation of measurable growth is delayed (Summary Graph Fig. 4.3). There is also a decrease in the amount of sporulation on visual examination, as the amount of lead increases.

4.3 Discussion

At concentrations up to 100 p.p.m. growth follows a similar pattern to the control. The regular increase in size is part of the normal pattern of growth since it is only the apical cells of the filaments which multiply (Brancato and Golding 1953). Even at high concentrations of lead once growth has begun it continues regularly so that the lead is not interfering with the normal pattern of growth except to decrease it. At 200 p.p.m. the amount of growth is lessened but there is no delay in initiation of growth and the growth is steady. It would seem that lead is only partially inhibiting growth and that the fungus was overcoming the effect of the poison to an extent. The amount of growth noticeably decreases at 300 p.p.m. when at the beginning growth is quite good and then it slows down, possibly due to the poison eventually penetrating the fungal metabolism to a sufficient extent to produce a significantly deleterious effect. In the early stages of growth the fungus may be using some stored food supplies so that the effect of the lead is not immediately evident. After the fourth day growth is more gradual so that the fungus is only just maintaining growth as measured by an increase in size. This pattern of growth is continued up to 600 p.p.m. although the amount of growth is decreasing. Between 700-1200 p.p.m. the initiation of measurable growth is delayed or proceeds very slowly until the sixth day when the amount of growth slightly increases. This may be due to the fungus nullifying or overcoming the effect of lead to a small extent.

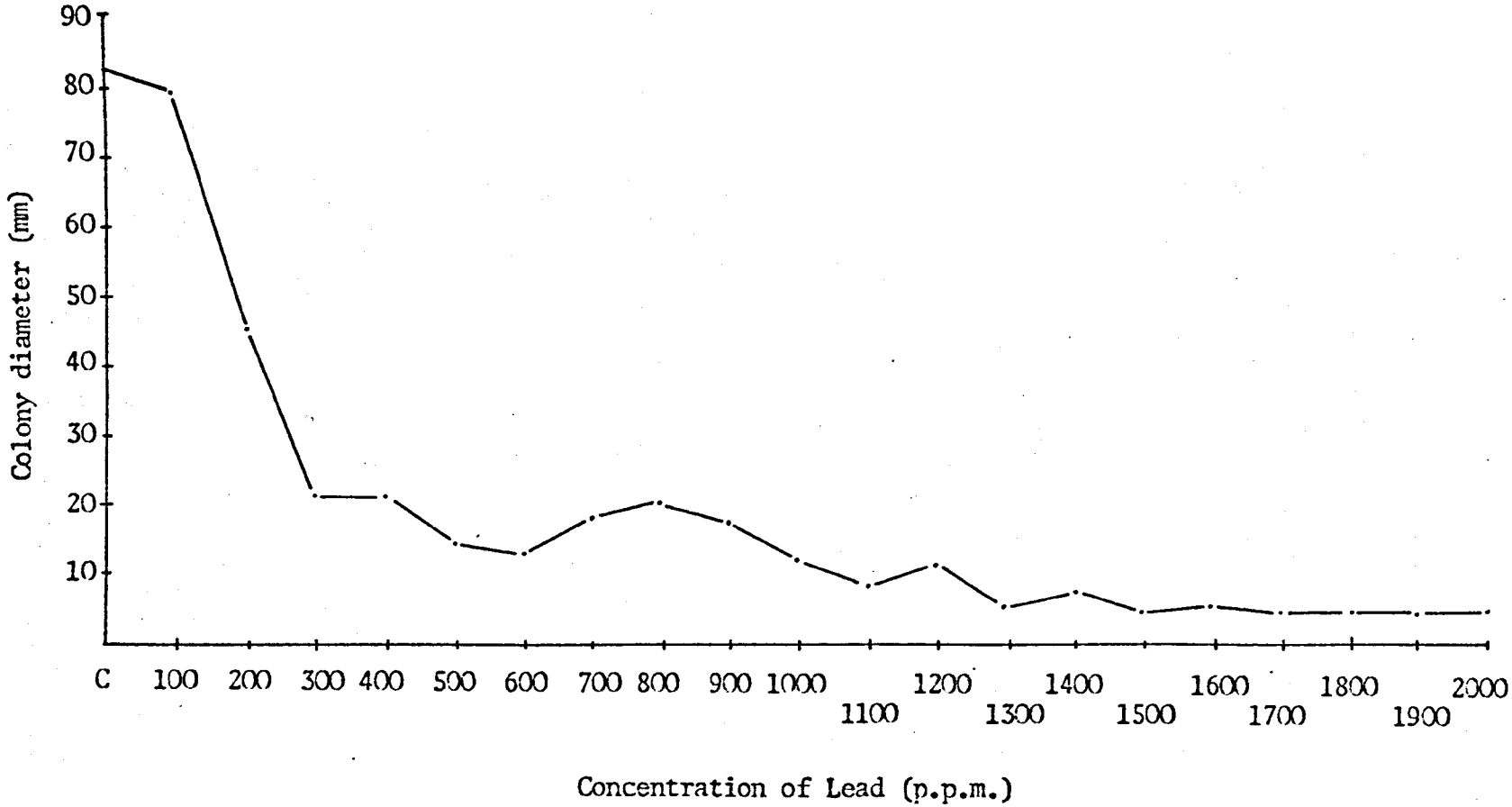


Figure 4.3

Colony diameter of A. fumigatus after eight days on various concentrations of Lead

At the higher concentrations there is very little growth sometimes only between the seventh and eighth day until there is no growth in eight days.

Lead is generally thought to exert its effect through the enzyme system as metals are often found to activate enzymes. At low concentrations of lead there is possibly not enough to affect the enzyme mechanism sufficiently to impair growth. It is able to lessen the amount of growth at slightly higher concentrations but it seems only to have a partial effect as a reasonable amount of growth is maintained. At still higher concentrations where the onset of growth is delayed or the amount of growth severely limited the amount of lead may be sufficient to inactivate the enzyme system entirely, but not to kill the organism. The reaction may continue in the fungus but at a very slow rate so allowing very little growth. An alternative to this possibility is that the fungus may be able to utilise another pathway so avoiding the affected system but the alternative may not be so efficient and so growth is not good.

In recent years studies have been made concerning other heavy metals in plants (Turner 1970) and fungi (Somers 1963). It has been found that the cell wall can accumulate heavy metals notably copper and zinc to some extent. This could have an effect on the ability to tolerate these metals by preventing some of the metal from reaching the cell metabolism. Somers found that the amount of copper accumulated in the cell wall of some fungi was higher than previously supposed. This could be important in that a certain amount of lead could possibly be inactivated in this way and this may be total accumulation up to 100 p.p.m. and beyond this concentration as the amount of lead increases so the amount affecting the cell metabolism rises. The discussion of these and other points will be continued in a later chapter.

There were some problems which arose during these experiments. First there was the problem of ensuring even distribution of lead nitrate within the agar as on addition there seemed to be some precipitation of a white substance. It was hoped that this would be lessened by using small quantities of lead and agar so enabling better mixing but the problem of precipitation is difficult. Also it is not known what is being precipitated and that loss of substance may affect the fungus adversely. Alternatively there may not be as much effective lead present as was thought.

Another problem arose due to the fact that Aspergillus fumigatus Fres. is a heavily sporing fungus (Plate 6) and although sporulation is lessened by the addition of lead it was still producing many spores. This means that often subsidiary colonies may develop and interfere with the colony which is being measured (Plate 5). This means that quantitative results may not always be very reliable as the colonies may be interacting before there is a visible interaction effect. This is a problem which is very difficult to solve as spores may fall from an inoculating needles or the slightest movement of the plate may spread them. As a result of these problems and the question of accuracy of results it was decided to continue experiments using liquid media and to discontinue solid media.

(a) Aspergillus fumigatus Fres. from lead mine spoil

PLATE 5

(b) Close up of A. fumigatus showing antagonism between colonies

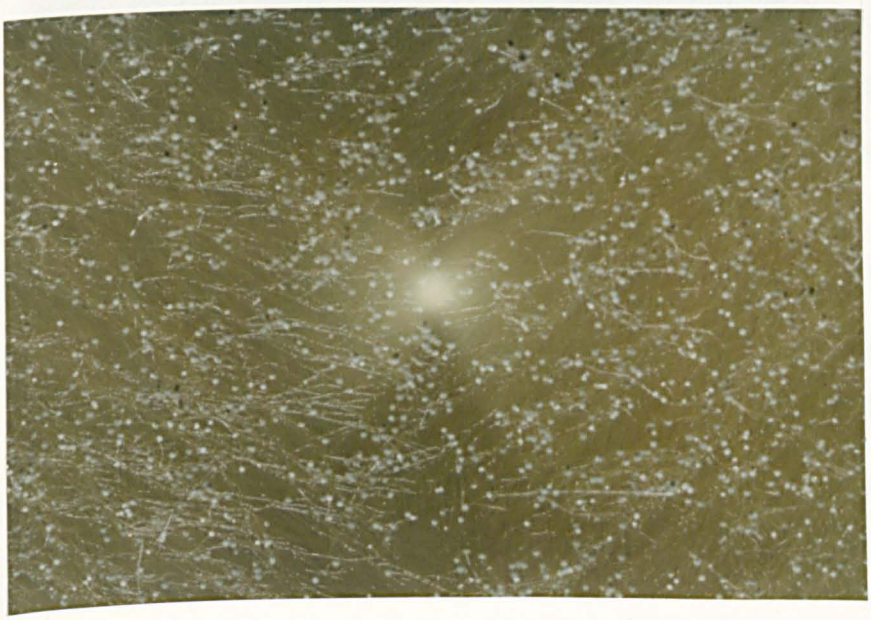


PLATE 6 Close up view showing thin mycelial growth and conidial heads of A. fumigatus

CHAPTER 5

LEAD AND THE GROWTH OF ASPERGILLUS FUMIGATUS FRES. AND SOME OTHER FUNGI IN LIQUID MEDIA

The work to be described in this chapter was done in liquid media as it has been shown previously that the use of solid media for present experimental purposes raised problems which were not easily solved. Liquid medium is generally considered a more accurate method by which to examine the growth of a fungus. Some of the problems of liquid culture were outlined by Cochrane (1958).

Before experiments could begin a suitable medium had to be found. Lead is a difficult metal to experiment with since many of its salts are insoluble and the addition of lead in a soluble form to a medium would deprive the fungus of necessary nutrients, particularly phosphorus with which the lead would combine (Zlochevskaya and Rabotnova 1966). This means that growth on such a medium would be affected by nutrient deficiency rather than by the direct effect of lead on the fungus. The amount of lead which might be acting on the fungus could vary as a result of factors other than the actual lead content.

Several natural media were tested and there was always some precipitation so that they were discarded not only for that reason but also because their chemical composition was often not known. Some synthetic media were tried and in each case a lead salt was precipitated. When individual constituents were tested most of the precipitation was associated with the component containing phosphorus. As all synthetic media seemed to have the same problem

Czapek Dox medium was selected for further experimentation. This medium was chosen since its chemical composition is known and also it supported good growth of Aspergillus fumigatus Fres. In this medium the precipitate was formed when lead was added to Magnesium glycerophosphate. Various alterations were made to the medium to try to prevent precipitation but the majority failed or presented another problem. However, one alteration was successful as it had been for Zlochevskaya and Rabotnova (1966). It is known that the solubility of some lead salts including lead phosphates, varies with pH of the solution. The solubility of lead phosphate increases as the solution becomes more acidic and with decreased pH and very large quantities of lead and magnesium glycerophosphate and complete Czapek Dox medium there was a decrease in the precipitate until none was visible at ca. pH 3.5-4.0. This seemed to be the answer so long as A. fumigatus could grow in a sufficiently acid medium to maintain the lead in solution. It was therefore decided to test the growth of A. fumigatus in media of different pH.

5.1 Growth of Aspergillus fumigatus Fres. in media of different pH

5.1.1 Experimental Procedure

The experiment was carried out using Czapek Dox (modified) medium, each flask contained 30 ml. The medium was acidified using Hydrochloric acid (A-R) and made more alkaline using Sodium hydroxide (A-R). The range of pH tested was:- 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8. The flasks were inoculated and incubated for ten days at 25°C.

5.1.2 Results and Discussion

The results are derived from the dry weight of the filtered mycelium from four flasks. The results are expressed in Table 79 and Fig. 5.1.

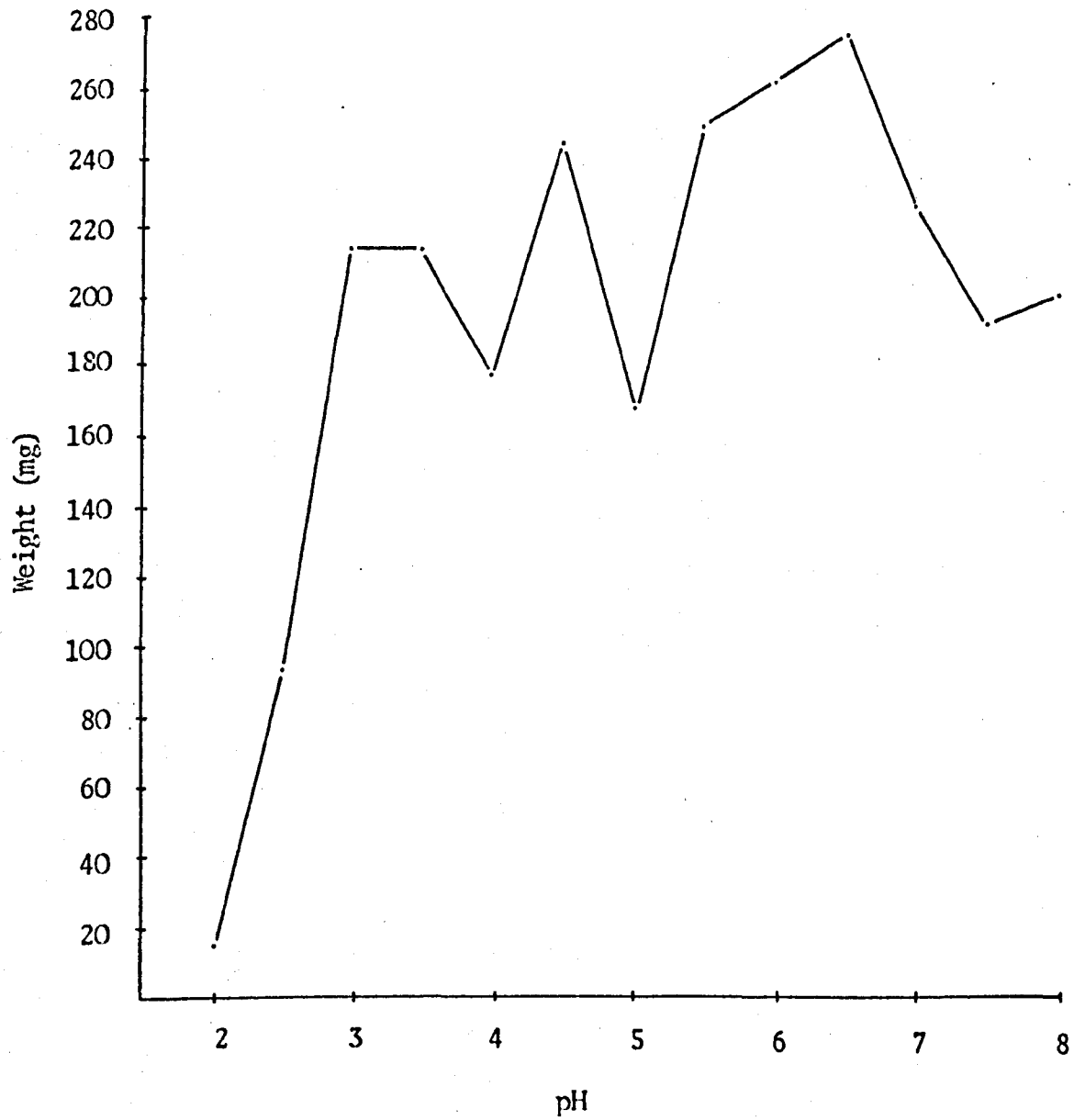


Figure 5.1

Growth of A. fumigatus in Czapek Dox liquid medium at a range of pH

From the graph it can be seen that most growth occurs around pH 6 and declines as the pH becomes more alkaline and acid. There is a distinct break at pH 3 below which growth becomes markedly affected by the level of acidity.

As a result of this experiment it was decided to conduct the experiments at pH 3 at which there was a considerable amount of growth of A. fumigatus. At this pH also there was no visible precipitation of lead salts and none was seen on microscopic examination of filter papers. This is slightly more alkaline than the medium used by Zlochevskaya and Rabotnova (1966).

5.2 Growth of Aspergillus fumigatus Fres. at pH 3 in a wide range of lead concentrations

This experiment was set up to ascertain the range of concentrations of lead which allowed growth of A. fumigatus and to determine the range for further experimentation. It must be emphasised that throughout this section all experiments were conducted at pH 3 and in some cases this may have affected the results, although this is not the opinion of the writer.

5.2.1 Experimental Procedure

Four replicate flasks were set up containing the following concentrations of lead:- 100, 150, 200, 250, 300, 350, 400, 500, 600 p.p.m. Four flasks containing no lead were used at each harvest. The flasks were inoculated and incubated for 4 or 16 days at 25°C.

5.2.2 Results and Discussion

The results are displayed in Table 80 and graphically in Fig. 5.2.

From the graph it can be seen that there was no growth above 200 p.p.m. It was therefore decided to conduct a more detailed

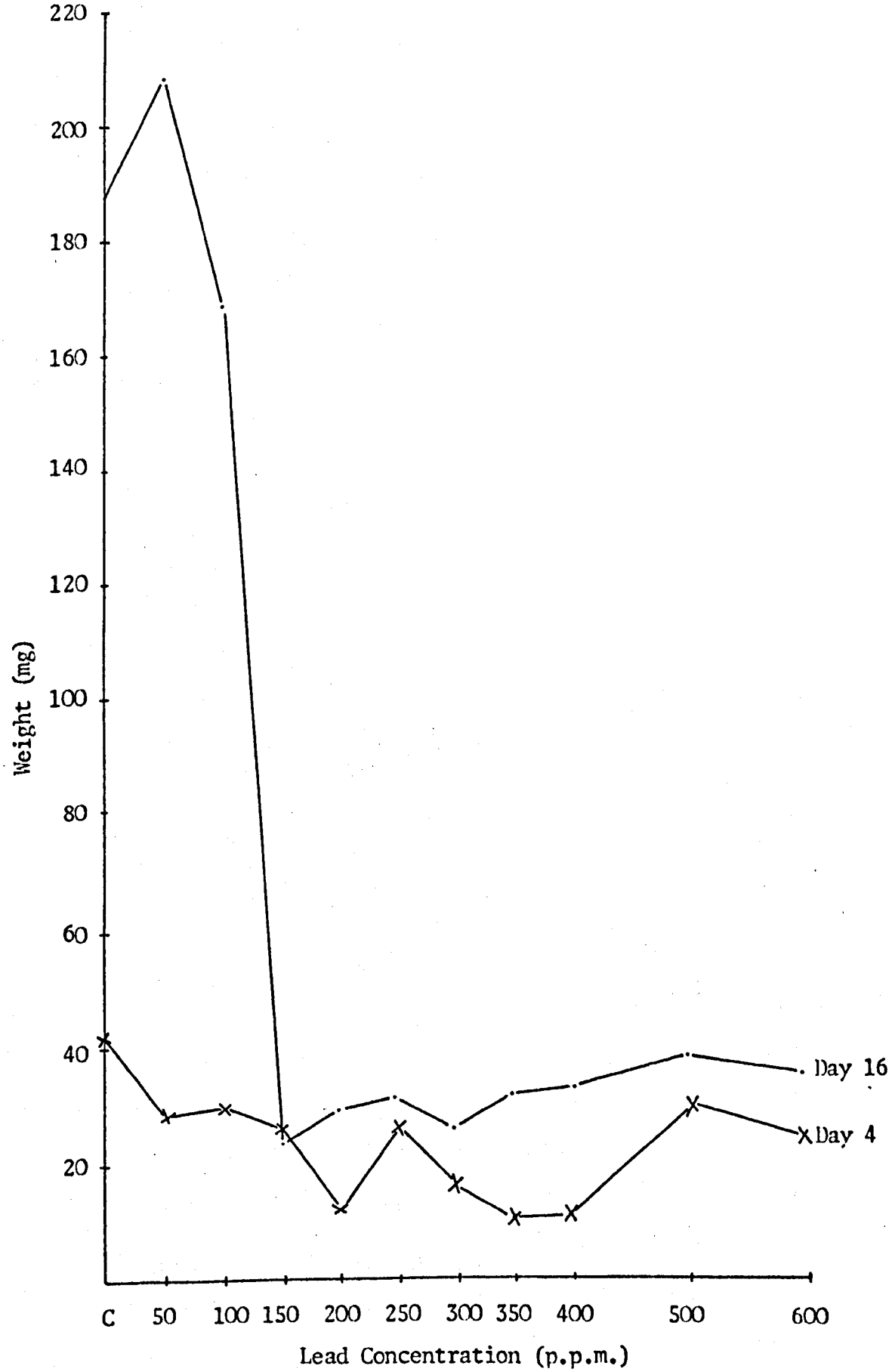


Figure 5.2

Growth of A. fumigatus in a range of Lead concentrations in Czapek Dox liquid medium

examination of the effect of lead up to a concentration of 220 p.p.m. on the growth of A. fumigatus.

5.3 Pattern of growth of Aspergillus fumigatus Fres. in various lead concentrations

As a result of the previous experiment it was decided to study the growth of A. fumigatus at a range of lead concentrations from 100 to 220 p.p.m. The experiment was designed to examine the amount and pattern of growth.

5.3.1 Experimental Procedure

Replicate flasks were set up containing 30 ml Czapek Dox medium. A set of control flasks contained no lead and the remaining flasks contained lead at the following concentrations:- 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210 and 220 p.p.m. Flasks were harvested every two days for 30 days. The flasks were inoculated with 1 ml of a spore suspension of A. fumigatus and put in a constant temperature room at 25°C. The results are expressed as the average weight of the mycelium in milligrans.

5.3.2 Results and Discussion

The results are presented in Table 81, Fig. 5.3 and Plate 7.

The general pattern of growth in the control and in the experimental flasks was one of a staling culture. There is a lag phase during which there is no apparent growth. In the control solution this phase only lasts for 4 days whereas at the lowest concentration of lead it is prolonged to 8 days which increases as the amount of lead increases, particularly at 150 p.p.m.

An analysis of variance conducted on the results after 6 days growth showed that only the control differed significantly

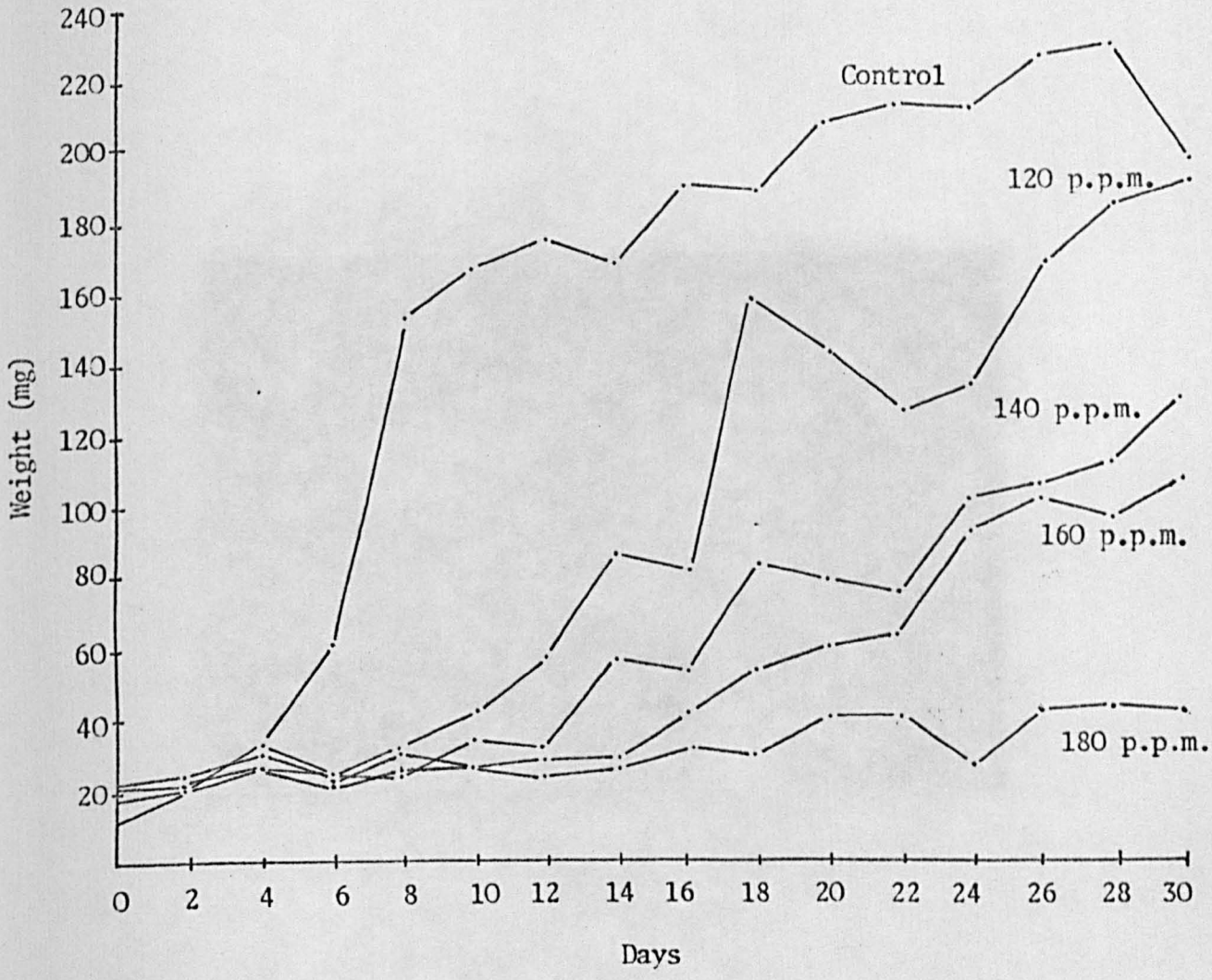
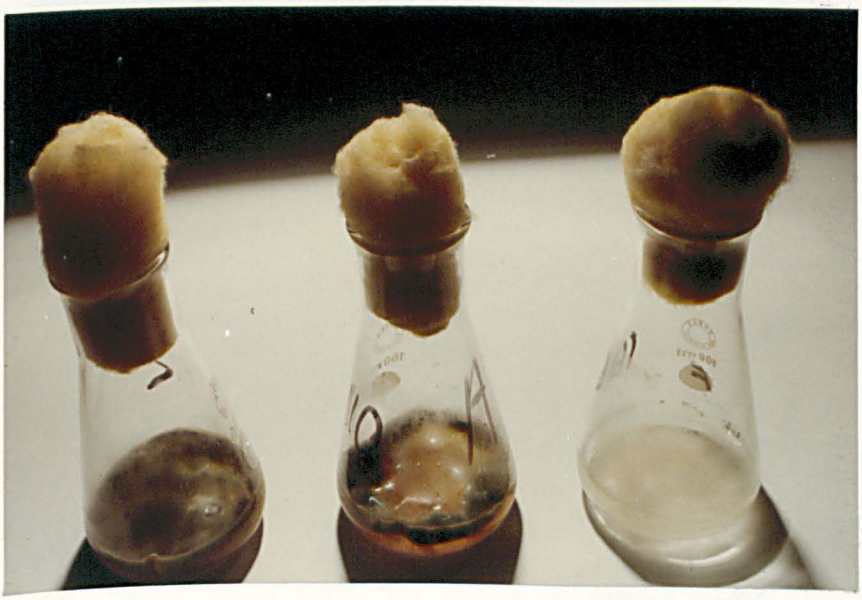


Figure 5.3

Pattern of growth of A. fumigatus in a range of Lead concentrations



Control | 110 p.p.m. Lead | 160 p.p.m. Lead

PLATE 7 Growth of Aspergillus fumigatus Fres. after 20 days in a Lead-free Czapek Dox medium and media containing 110 and 160 p.p.m. Lead

from the rest. There was little variation in the amount of growth in media containing different concentrations of lead. At day 8 there is a similar pattern, however, there are considerable differences between the flasks containing 100 p.p.m. of lead giving rise to a significant variation. This suggests that growth is beginning at this concentration. There is then a phase of growth where the size of the colony increases very rapidly. After 18 days there are significant differences between all the concentrations of lead and also the control indicating a real effect on growth of all the media. The speed and extent of growth in this period decreases as the lead content increases until at 170 p.p.m. growth is only gradual and there is no marked change from the lag phase to the second phase. After 30 days the difference between the concentrations is reduced although it is still statistically significant. There is some indication that there are two groups arising from the different media. One group, with low concentrations of lead, are approaching the total amount of growth shown by the control. The other group allow only limited growth of the fungus. It may be that there would eventually be a distinct difference between the two groups. However, the statistics show the variation in the pattern of growth at the different concentrations. There seem to be several critical concentrations of lead where there is a marked change in the pattern of growth, such changes occurring between 120-130 p.p.m. and 160-170 p.p.m.; beyond 170 p.p.m. there is no growth which can be shown by weight.

At the higher concentrations of lead (140 p.p.m. +) there is not such an obvious slowing off in growth rate, although the total amount of growth is reduced. The implication here is that since the media can support more growth so it is the concentration of lead which in effect is having the detrimental effect. There may be a slight slowing of growth due to the fact that during the growth period the

amount of water in the medium decreases so that the amount of lead increases and this might have an adverse effect on the growth of the fungus in the later stages. It is difficult to know whether growth at these high concentrations would eventually equal the control (this would have to be tested in a medium of constant composition).

Once the lag phase is finished growth proceeds in all concentrations up to 170 p.p.m., but there is a clear effect of the lead concentration on the rate of growth as this is also slowed down at high lead levels. Thus, there is evidence of a three-sided effect, firstly an increased delay in the inception of relatively rapid growth, secondly a reduction in rate of growth proportional to the lead concentration and thirdly a delay in the initiation of what could be termed the staling phase, for although the colonies at high lead concentrations were small they were still growing actively in contradistinction to the control which had ceased to grow by the 28th day. It would be interesting to examine the effect of lead on the growth pattern of the fungus after initiation of growth with regard to the total amount of growth and the rate of growth.

Heavy metals are believed to act at the surface of cells complexing with the active anionic sites (Horsfall 1956) and producing changes in the surface tension and surface charges of the lipid films at the cell wall (Passow, Rothstein and Clarkson 1961). This affects the permeability of the membrane, which in low concentrations of lead is not affected as it is at higher concentrations where the membrane may be so disrupted that no growth is possible. At low concentrations smaller areas of the cell wall may be affected so that although permeation of nutrients occurs it is at a reduced rate so slowing growth but not preventing it. The effectiveness of the various concentrations of lead may also be a function of the ability of the fungus to complex lead with non-vital

components of the cell and so effectively remove it from the metabolism of the cell. Thus the amount affecting the cell is reduced, although this method may not be so efficient that all the lead even at the lowest concentrations used is prevented from having some effect on the cell. If the fungus needs initial build-up of nutrients then with reduced permeation or reduced activity of enzymes attacking the external nutrients to render them suitable for absorption the onset of growth would be delayed but would occur eventually, although the rate would still be slower than the control because of the reduced efficiency of the cell membrane. As the amount of lead increases, so the amount of disturbance increases until eventually the fungus cannot grow at all.

Although much of the heavy metal activity is generally associated with the cell wall there is usually some penetration into the cell. Once inside the cell wall there are many substances to which heavy metals can bind and so affect the metabolism of the cell. Heavy metals can affect enzymes by interfering with the active site in some way, however, the effectiveness of the metal depends on the accessibility and functional significance of the groups binding the metal (Passow et al. 1961). This might give rise to a reduced amount of growth of the fungus since the enzyme may be active but at a reduced level. Eventually the cell may be able to inactivate some of the lead and then grow at a faster rate which might explain the long lag phase. Alternatively the cell may be able to manufacture more enzyme so that the reaction may continue at a faster rate.

Heavy metals are known to disrupt cells in many ways resulting in reduced growth or no growth and further investigation is necessary to elucidate the mode of action of lead on A. fumigatus. It may be that if lead produces many effects which are largely unspecific there will be a reduction in growth and finally no growth at all as lead

concentrations are increased. In such a case no immediately obvious effect would be noticeable.

5.4 The effect of lead on the germination of Aspergillus fumigatus Fres. spores

Most of the experimental studies with heavy metals and other substances, many of which are fungicides, have been conducted using spores and their germination (Somers 1963, McCallan and Wilcoxon 1934, McCallan, Miller and Weed 1954). For this reason and also to examine the effect on germination in relation to the lag period, it was decided to conduct this experiment.

5.4.1 Experimental Procedure

The hanging drop technique was used as described in Chapter 1. Three types of drop were set up. Two contained spores suspended in distilled water but one set had the pH adjusted to 3 using hydrochloric acid. In the other type of drop the spores were suspended in Czapek Dox liquid medium with the pH adjusted to 3. The following concentrations of lead were present in the drops (in p.p.m.):- 110, 150, 170, 180, 190, 200, 210, 220, 250, 300 and 400. Controls containing no lead were set up for each kind of drop, which were then incubated at 25°C.

5.4.2 Results and Discussion

The results are presented in Table 5.1 and Figs. 5.4 and 5.5.

Although there are several stages in the germination process of A. fumigatus conidia (Campbell 1971) for convenience the emergence of the germ tube is taken as the beginning of true germination.

There was a difference in the behaviour of the conidia in the control drops of distilled water. In the drop of normal distilled water 73% of spores germinated and germ tubes of 51μ in length were produced in 24 hours. Conidia of A. fumigatus can germinate relatively

well without external nutrients. In the drop of distilled water at pH 3 germination did not occur until between the second and third day. After three days 70% of spores germinated with germ tubes of 47μ in length. The acidity of the distilled water does not prevent germination but does delay it markedly. It does not seem to have much effect on the rate of growth of the germ tubes. Cochrane (1958) considers that pH 3 is the lower limit at which germination occurs, the optimum range being pH 4.5 - 6.5. However, the addition of nutrients nullifies the delaying action of an acid pH since in Czapek Dox of similar pH there was 80% germination in 24 hours. In fact the percentage germination has increased slightly.

The addition of lead to both the distilled water drops prevented any germination of A. fumigatus conidia over a period of seven days. It seems that either the spores are unable to germinate in the lead solutions or that germination is very markedly delayed. It is impossible to estimate the percentage germination or amount of growth in the drops of distilled water without lead after seven days since the germ tubes had grown to such a length that they had become interwoven and felted forming a distinct colony, thus effectively preventing the examination of individual spores.

Germination did occur in the Czapek Dox drops containing lead and a graphical representation of the results is presented in Fig. 5.4. The control drop contained 80% germination in 24 hours and was totally overgrown in 48 hours.

In the solutions containing 110 and 150 p.p.m. of lead germ tube emergence occurred between 24 and 48 hours, so that by day 2 most of the spores had germinated, this is one day after the control. The lead is not having much effect in these solutions it would seem.

Table 5.1

% germination in a range of lead concentrations over 6 days and germ tube length after 3 and 6 days

	Days						Germ tube length (μ)	
Solution	1	2	3	4	5	6	after 3 days	6 days
Control	80	99	x	x	x	x		
110		90	x	x	x	x	84	
150		86	90	x	x	x	30	
170		69	90	x	x	x	15.6	
180			33	90	x	x	12	
190			33	55	90	x	11	
200			13	54	90	x	11	
210			14	49	89	x	13	
220			16	51	87	x	10.6	
250			14	48	88	x	7.7	37
300				10	17	26		22
400*								

x - most spores germinated, no estimate possible because of clumping and hyphal growth.

* - Swelling only - no germ tube emergence.

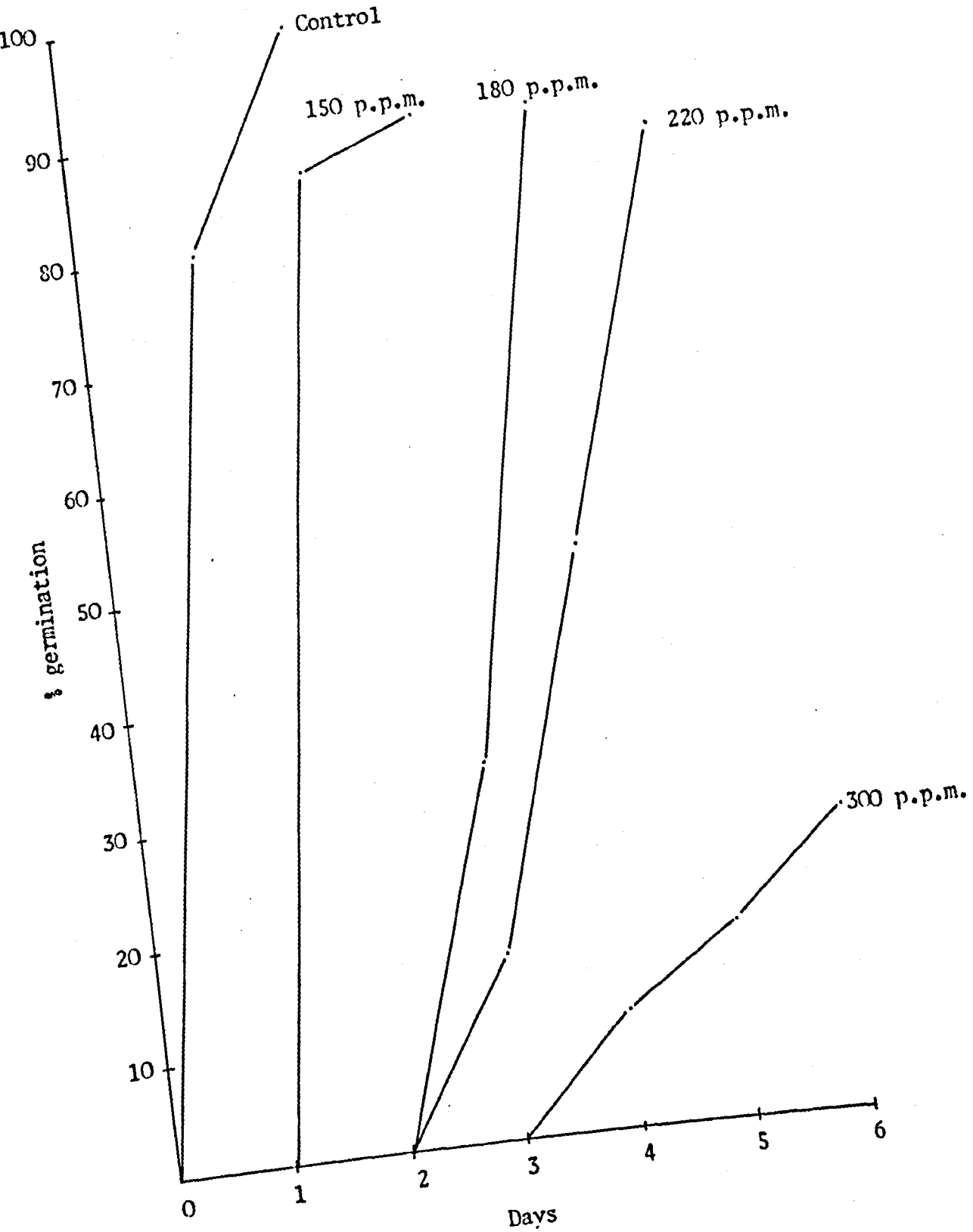


Figure 5.4

% germination in hanging drops of Czapek Dox liquid medium containing different Lead concentrations

Germination was first observed on the second day in the solutions containing 170, 180 and 190 p.p.m. of lead and continued at varying speed so that by day 3 the amount of germination was not the same in each solution, although the onset was the same.

In solutions containing 200, 210, 220 and 250 p.p.m. of lead, germ tube emergence was first seen on day 3 and growth and germination continued from then. However, there was variation in the extent to which germination had proceeded on day 3.

By day 4 10% of the spores in 300 p.p.m. had germinated and after 6 days 26% of the spores had germinated so that there is little increase in the numbers of spores germinated. At 400 p.p.m. only swelling of the spores was observed and although this is the first stage in germination there was no emergence of a germ tube even after 8 days and it would seem unlikely to occur later than this since the swelling was observed on the first day.

After 6 days, germination was almost total at all concentrations below and including 250 p.p.m. The spores which had not germinated were probably non-viable rather than affected by lead since the number did not vary appreciably at any lead concentrations. Observation using a light microscope showed no physical differences between the germinating spores in the control drops or the lead containing drops.

The present investigation confirms Campbell's (1971) statement that germination of A. fumigatus conidia is not entirely dependent on external nutrients, the addition of which does stimulate the rate and number of spores germinating. Campbell (1971) suggests that a non-nutrient factor, such as the presence of water, triggers off the germination process. Increased acidity obviously affects this in some way delaying the reaction, although the situation can be returned to the considered norm by the addition of nutrients. The acidity does

not lead to a complete dependence on nutrients but rather an improved performance when they are present. This does not necessarily suggest that the triggering mechanism is not non-nutrient. Acidity on the other hand may affect reactions occurring in the conidia by which necessary products are produced, the addition of nutrients may simply supply these products or shorten pathways of production.

Lead may interfere more fundamentally with the germination process since there was no germination at all without the presence of nutrients. The lead may act at the cell surface, a site where heavy metals accumulated in spores (Somers 1963), so hindering the factor in its effect on the spore, and where there are no nutrients the spores are unable to respond. This is unlikely to be the major effect of lead in the solutions containing nutrients since swelling was observed on the first day at all concentrations. The delay in germ tube emergence in the later stages is much more apparent. Since germination in the presence of lead seems to be dependent on external nutrients it would seem that the lead is probably blocking fundamental metabolic pathways which produce substances necessary for germination. However, this interference can be overridden in some way since there is eventual germination at quite high concentrations. During germination Campbell (1971) suggests that lipid bodies may be used as nutrients since they seem to disappear and it may be that the lead interferes with the use of these in some way so making the spore dependent on external nutrients. In Aspergillus niger conidia there is a build up of certain enzymes before germ tube emergence (Burnett 1968) which could be affected by lead to the extent that emergence is delayed or prevented.

The effect of lead need not be any one of these possibilities singly but perhaps a combination of many reactions, since there are

many active sites with which the lead can complex and upset the metabolism. In the drops without nutrients all concentrations of lead are effective in preventing germination but in the drops containing nutrients the lead only delays germination at low concentrations, but is able to prevent it at a much higher concentration. To explain the effect of lead it would be necessary to conduct further investigation possibly using labelled lead similar to those using labelled copper (Somers 1963) to examine where the lead is effective, which might indicate how it was acting. However, it may prove similar to copper which can complex with many cell fractions so giving little indication of specific action.

In a consideration of the growth of the germ tube after its emergence it would seem that it is more affected by lead in the solutions than is germination. In Fig. 5.5 the average length of the germ tubes is shown after three days and despite the high percentage of germination at the lower concentrations only 110 p.p.m. shows marked growth. It must be remembered that the time of emergence affects the growth since the longer time since emergence then the more growth should have occurred. However, with concentrations with similar initial germination times it would be expected that germ tube growth would be similar, but there are marked differences, for example the growth of the germ tubes in solutions with 110 and 150 p.p.m. A subjective examination of the amount of growth of the germ tube after 6 days in concentrations of 180 to 250 p.p.m. showed there was little growth. Measurement in this case was difficult since the spores had clumped in the bottom of the drop. This may also affect the growth of the germ tubes by interference, but the lead is also active as is shown in the earlier stages before the spores are so close.

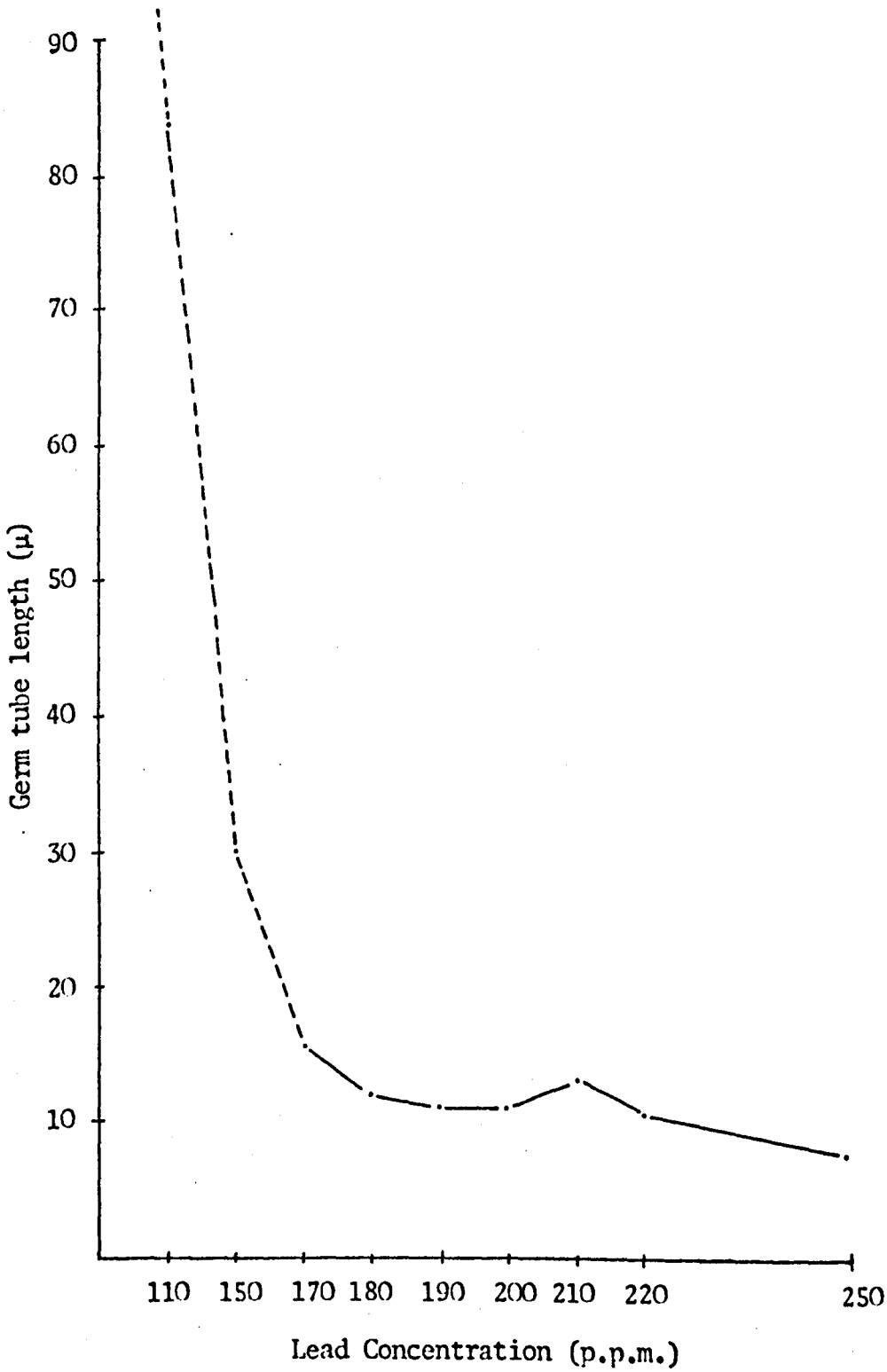


Figure 5.5

Germ tube length after 3 days in hanging drops of Czapek Dox liquid medium containing different Lead concentrations

Lead, therefore, affects germination but it influences germ tube growth much more since germination increases quite well eventually, but at the higher concentrations the growth of the germ tube does not. The spore may be protected by its outer coat which is supposed to be fairly resistent but the germ tube wall consists only of the inner layer of the spore and so may be more susceptible to the action of lead.

5.5 The timing of the effect of lead on Aspergillus fumigatus Fres.

It was decided to investigate whether lead affected the fungus at any particular time or whether it exerted its effect throughout the growing period since growth is prevented at a lower concentration than that which prevents germination. Further, Zlochevskaya and Rabotnova (1968) found that various compounds of lead decrease in toxicity to Aspergillus niger as the culture grew.

5.5.1 Experimental Procedure

Ninety-six flasks containing 29 ml of Czapek Dox liquid medium at pH 3 were inoculated with a spore suspension. After one day eight of the flasks were filtered and weighed. The contents of four flasks were transferred to four fresh flasks containing Czapek Dox medium only at pH 3. The contents of the other four flasks were transferred to four fresh flasks containing similar Czapek dox medium and lead at a final concentration of 170 p.p.m. This process was repeated after 2, 4, 6, 8 and 10 days. Pouring was adopted as the method of transfer since there was little visible growth in the early stages of the experiment. The flasks were then incubated at 25°C for 20 days.

5.5.2 Results and Discussion

The results are presented in Table 5.2 and Fig. 5.6.

The amount of growth was greater than in other experiments,

Table 5.2

Growth of Aspergillus fumigatus at transfer and after 20 days in lead-containing medium and control solutions

DAY	MEAN WEIGHT (in mg)					TOLERANCE FACTOR (a-b)	t test
	Weight at Transfer	FINAL WEIGHT		GROWTH			x-p=0.05 xx-p=0.01
		in Pb solution	in Pb-free solution	in Pb (a)	in Pb-free (b)		
1	14	112	274	98	260	-162	x
2	14	220	331	206	317	-111	xx
4	36	310	271	274	235	+39	x
6	165	374	393	209	228	-19	N.S.
8	155	414	382	259	227	+32	x
10	174	356	385	182	211	-29	N.S.

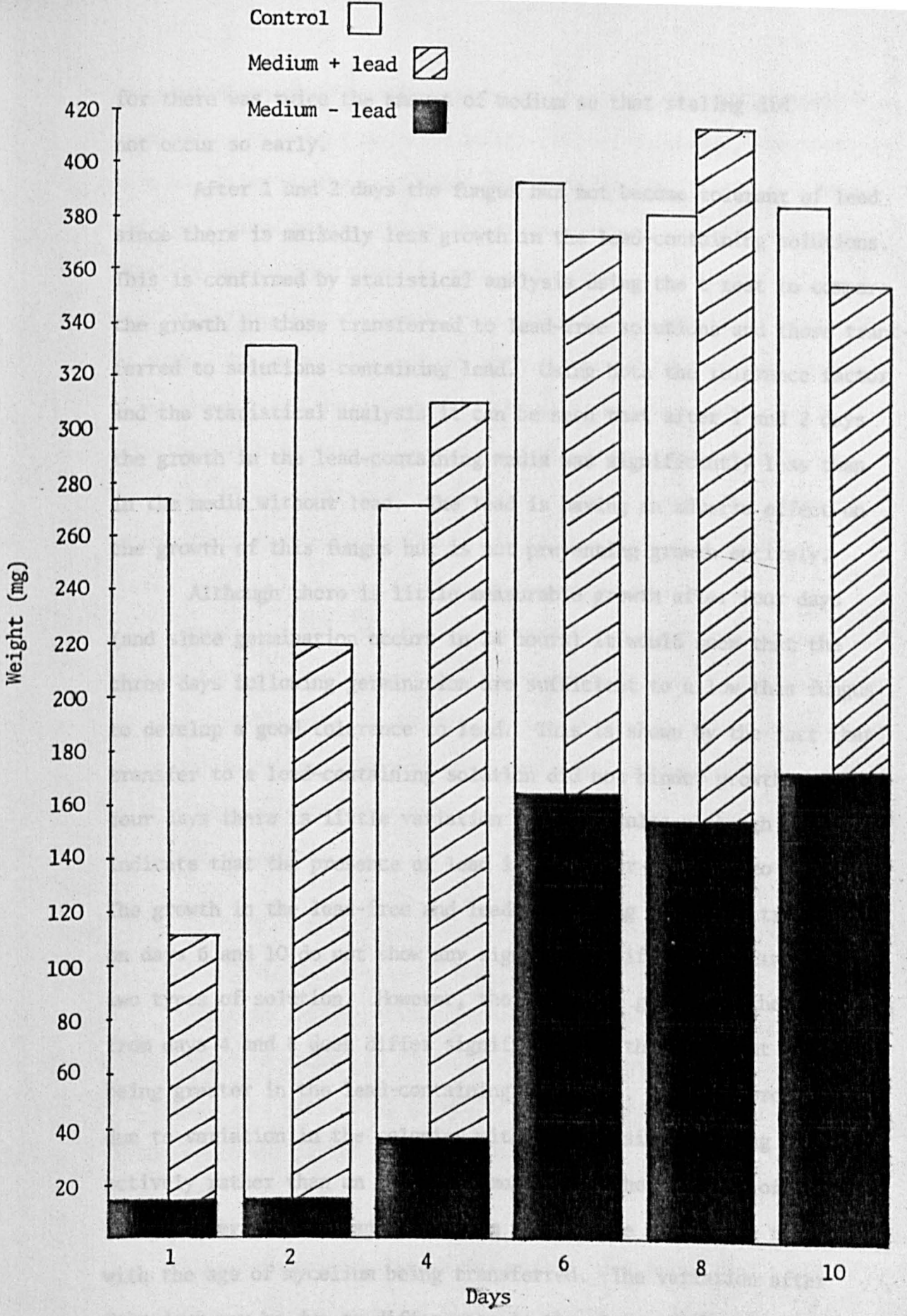


Figure 5.6

Growth of A. fumigatus in lead-free solutions and then transferred to 170 p.p.m. lead solutions and in lead-free solutions throughout

for there was twice the amount of medium so that staling did not occur so early.

After 1 and 2 days the fungus has not become tolerant of lead since there is markedly less growth in the lead-containing solutions. This is confirmed by statistical analysis using the t test to compare the growth in those transferred to lead-free solutions and those transferred to solutions containing lead. Using both the tolerance factor and the statistical analysis it can be seen that after 1 and 2 days the growth in the lead-containing media was significantly less than in the media without lead. The lead is having an adverse effect on the growth of this fungus but is not preventing growth entirely.

Although there is little measurable growth after four days (and since germination occurs in 24 hours) it would seem that the three days following germination are sufficient to allow this fungus to develop a good tolerance to lead. This is shown by the fact that transfer to a lead-containing solution did not hinder growth. After four days there is little variation in the results although they all indicate that the presence of lead is no longer inimical to growth. The growth in the lead-free and lead-containing solutions transferred on days 6 and 10 do not show any significant difference between the two types of solution. However, the amount of growth in the solutions from days 4 and 8 does differ significantly with the amount of growth being greater in the lead-containing solutions. This is probably due to variation in the colonies with some possibly growing more actively rather than an active stimulus from the presence of lead.

Tolerance to lead would seem to increase with time, that is with the age of mycelium being transferred. The variation after four days may be due to differences in the state of the mycelium at transfer, or possibly a different rate of spore production which

on germination would be affected by the presence of lead. Since germination will have occurred in the control solutions, the lead must be acting on the germ tubes and young hyphae following transfer and since heavy metals are active at the cell surface (Horsfall 1956) it may be that there is some difference, e.g. in the cell wall or plasma membrane which makes young hyphae specially susceptible to lead. Alternatively lead may enter young cells more easily than older growth. If the effect is internal some vital system may be affected which is more necessary to young growth than to older mycelium. Lead could affect the production of a specific substance manufactured in the young hyphae but if the substance can be recycled lead could be ineffective in the older colonies.

Generally the attack of heavy metals is relatively unspecific so that further experimentation is necessary to clarify the situation and the possible site of action. An investigation of any differences in structure or physiology of young and older hyphae would be useful, as this may indicate areas which would seem possible sites of activity for further study.

5.6 Pattern of growth in 170 p.p.m. lead after 6 days growth in control solution of Aspergillus fumigatus Fres.

After an initial investigation indicating that lead was not effective after growth in control solution it was decided to investigate the pattern of growth before and after transfer to see if there was any period of adjustment.

5.6.1 Experimental Procedure

Flasks containing Czapek Dox medium were inoculated. After 6 days growth the contents of half the flasks were transferred to flasks containing medium and no lead. The contents of the remaining

flasks were transferred to flasks containing medium and lead at a concentration of 170 p.p.m. Some of the flasks were harvested every two days. The flasks were incubated at 25°C.

5.6.2 Results and Discussion

A normal growth curve of a staling culture resulted in this experiment (Fig. 5.7). However, after transfer the control solution had a marked increase in growth unlike the medium containing lead where the fungus grew more gradually. In effect it would seem that the log phase of growth is lengthened in time by the presence of lead, although the total amount of growth is similar to the control solution. After the log phase the growth rate lessens probably due to a decline in the available nutrients.

There is no indication of a period of adjustment by the fungus to the presence of lead since growth continues albeit slower than the control. It seems that a 6 day old mycelium under normal conditions contains some mechanism which reduces the undoubted toxicity shown by lead towards young mycelium. This is an important conclusion, since it demonstrates that the fungus is not developing a lead tolerance, but by 6 days is, in fact, naturally tolerant of lead. It may be, therefore, that the absence of fungal mycelium in lead contaminated soils is not a consequence of the inability of the mycelium to grow, but rather of spores to germinate and growth to get started in these situations.

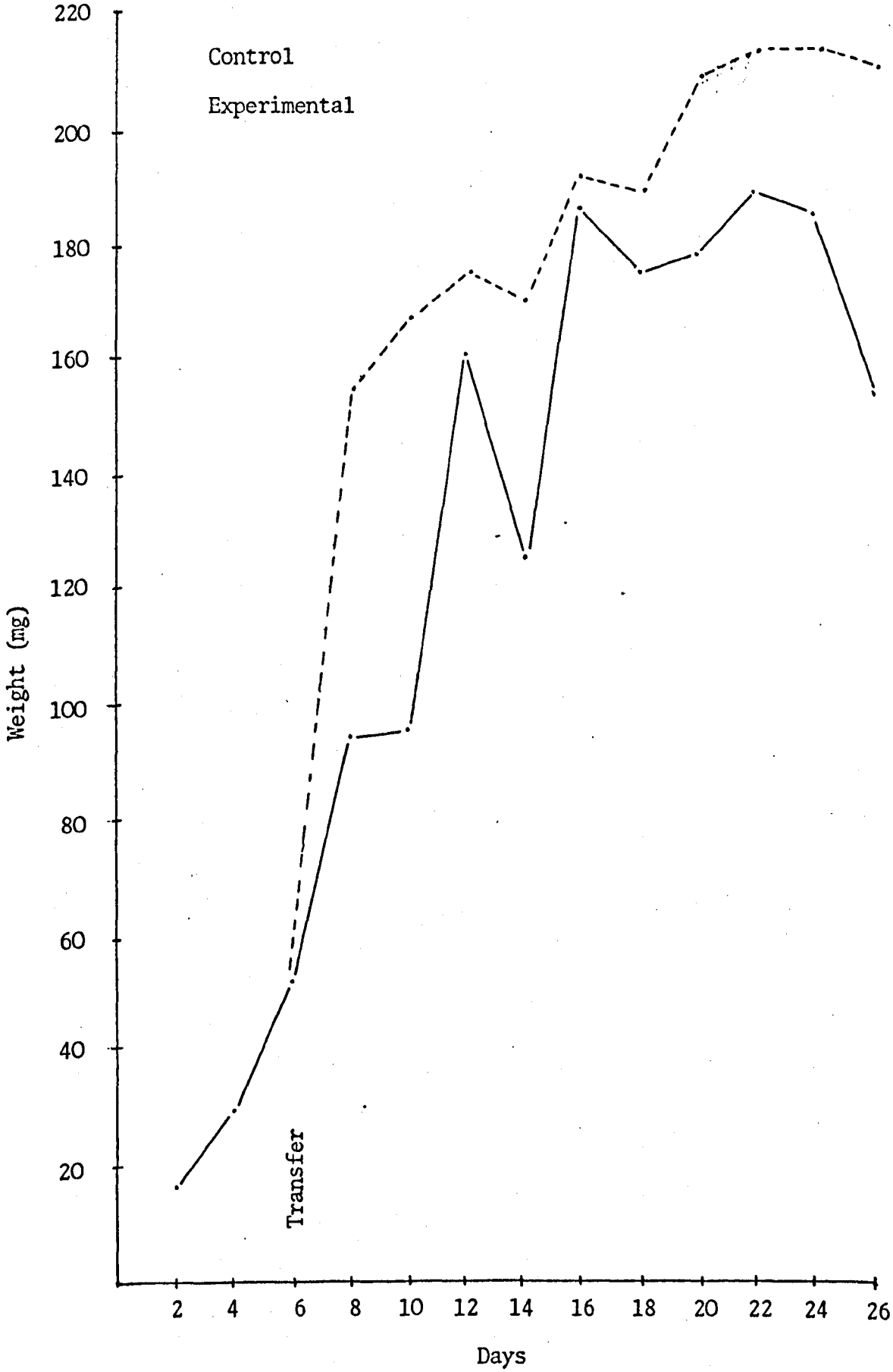


Figure 5.7

Growth of A. fumigatus in a lead-free solution transferred to 170 p.p.m. lead after 6 days and lead-free solution throughout

5.7 Growth of Aspergillus fumigatus in control (lead-free) solutions following initial growth in lead solutions

Zlochevskaya and Rabotnova (1968) showed that the effect of lead on Aspergillus niger seemed to be 'bacteriostatic' rather than 'bacteriocidal'. It was decided to investigate if the effect on A. fumigatus was permanent or if it could be reversed or nullified by transfer into control solution.

5.7.1 Experimental Procedure

There were two kinds of flasks of Czapek Dox medium. To one set lead was added to a concentration of 170 p.p.m. The flasks were inoculated. After four days the contents of four of the flasks without lead were filtered using a millipore filter (0.2μ). The filter and residue on it were then transferred to flasks containing fresh medium. The contents of eight of the lead-containing flasks were also filtered in the same way. Four of the filter papers were then placed in lead-free flasks and the other four in flasks containing 170 p.p.m. of lead. Eight of the original flasks, half containing lead, were filtered and the contents weighed. This process was carried out after 6, 8 and 10 days. The flasks were incubated at 25°C for 20 days.

5.7.2 Results and Discussion

The results are presented in Table 82 and Fig. 5.8.

Growth in the controls was quite good, particularly those containing lead. In the flasks without lead the colony grew on the surface as normal, however in the lead-containing controls growth was associated with the millipore filters. Growth in lead-containing solutions in previous experiments usually began on the wall of the flasks and this was also true of Botrytis cinerea in

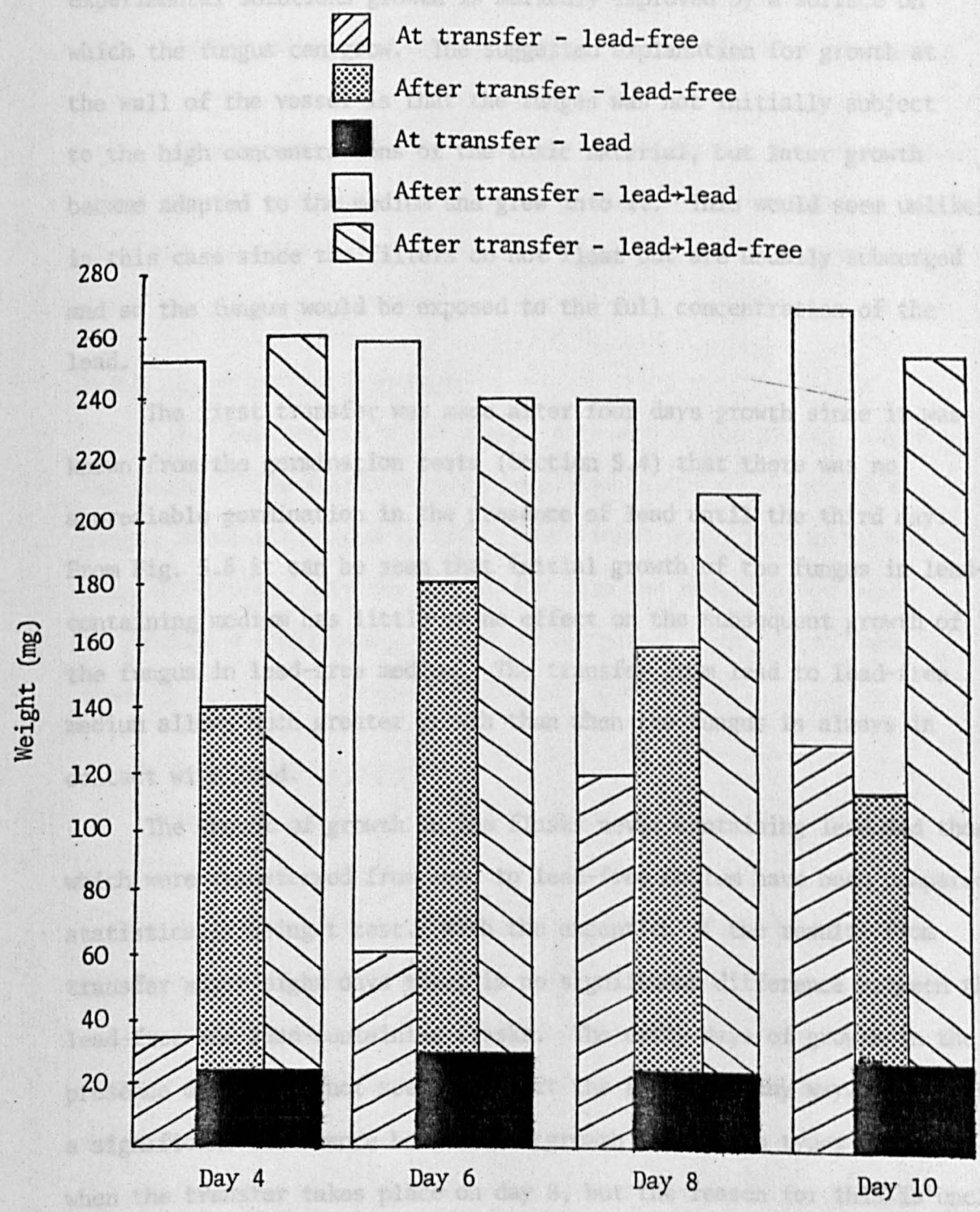


Figure 5.8

Growth of A. fumigatus in 170 p.p.m. lead, lead-free solutions and 170 p.p.m. lead solutions then transferred to lead-free solutions

copper solutions (Parry and Wood 1958) and growth on the filter may be an extension of this. It would seem that the fungus does not need a solid surface for good growth in control solutions, but in experimental solutions growth is markedly improved by a surface on which the fungus can grow. The suggested explanation for growth at the wall of the vessel is that the fungus was not initially subject to the high concentrations of the toxic material, but later growth became adapted to the medium and grew into it. This would seem unlikely in this case since the filters do not float but are usually submerged and so the fungus would be exposed to the full concentration of the lead.

The first transfer was made after four days growth since it was known from the germination tests (Section 5.4) that there was no appreciable germination in the presence of lead until the third day. From Fig. 5.8 it can be seen that initial growth of the fungus in lead-containing medium has little or no effect on the subsequent growth of the fungus in lead-free medium. The transfer from lead to lead-free medium allows much greater growth than when the fungus is always in contact with lead.

The amount of growth in the flasks never containing lead and those which were transferred from lead to lead-free medium have been compared statistically using t test. With the exception of the results from transfer after eight days there is no significant difference between the lead-free and lead-containing flasks. The early days of growth in the presence of lead do not seem to affect the fungus in any way. There is a significant difference between the growth in the two types of media when the transfer takes place on day 8, but the reason for this is unclear since a significant difference is not apparent on day 10. It may therefore be due to experimental error. It would seem that the

effect of lead is fungistatic rather than fungicidal, since any effect the lead has on the growth of A. fumigatus does not prevent good growth in the lead-free solutions.

5.8 Growth of Aspergillus fumigatus in lead solutions containing one amino acid.

Since amino acids are important metabolically and also contain sites capable of binding substances to them, it was decided to see if they had any effect on the growth of A. fumigatus in the presence of lead.

5.8.1 Experimental Procedure

Two hundred and eight flasks were set up containing Czapek Dox medium. The flasks were divided into groups of eight and a single amino acid added to each group except one. Each group was then sub-divided into two halves and lead was added to one half to produce a final concentration of 160 p.p.m. The flasks were then inoculated and harvested after 20 days at 25°C.

5.8.2 Results and Discussion

The results are expressed in Table 83 in the Appendix and some of them are presented in Fig. 5.9. Statistical analyses using t test were carried out on the results comparing growth in the presence of lead with growth when lead is absent.

In the control solutions containing no lead there is not much variation in the amount of growth, nor do the media containing amino acids grow appreciably more than the control solution of Czapek Dox solution. It would seem that on the whole A. fumigatus can utilise sodium nitrate as efficiently as individual amino acids and their presence does not normally give rise to very much increased growth.

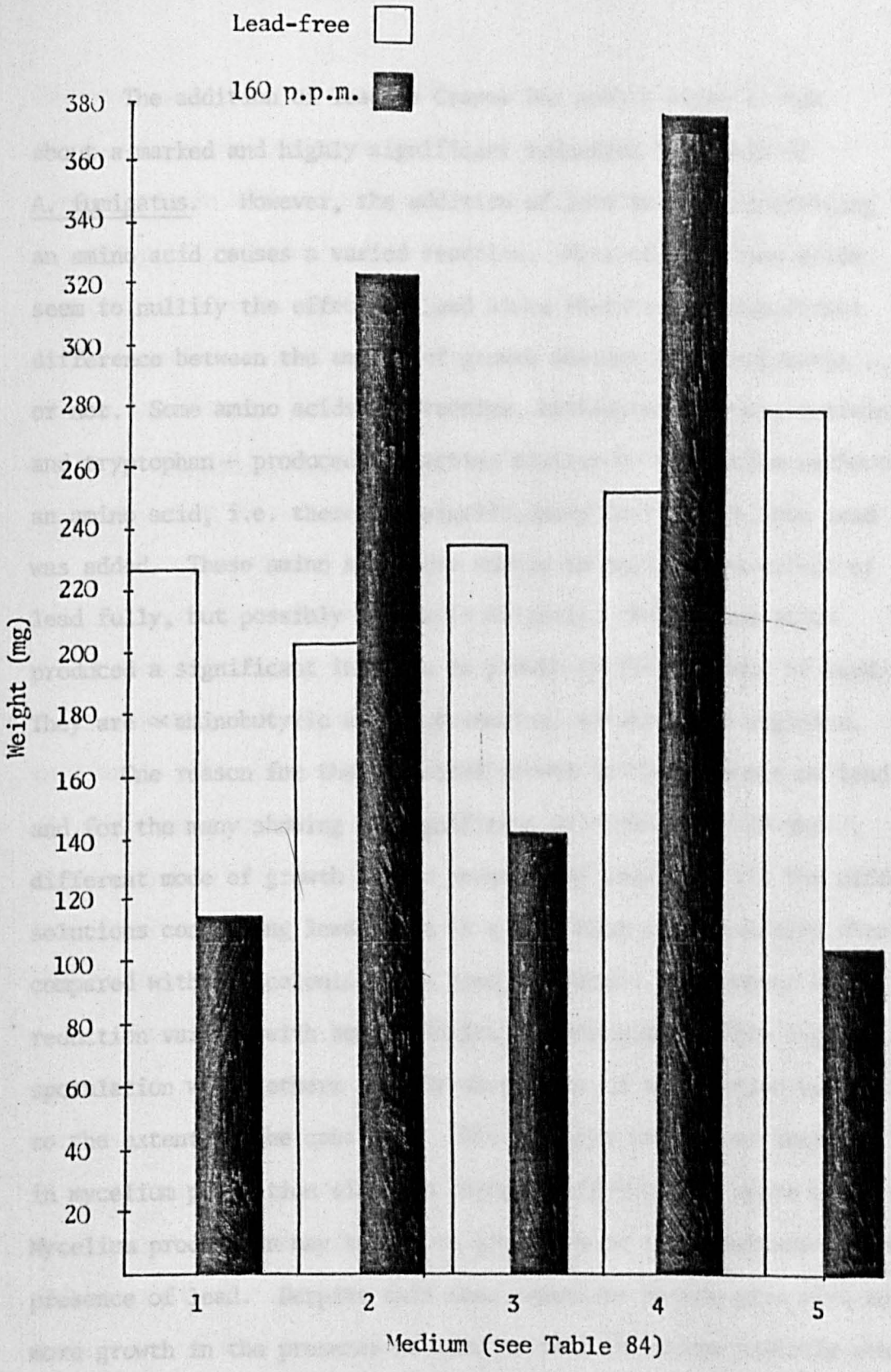


Figure 5.9

Growth of A. fumigatus with some single amino acids added in lead-free media and media containing 160 p.p.m. lead

The addition of lead to Czapek Dox medium alone brings about a marked and highly significant reduction in growth of A. fumigatus. However, the addition of lead to media containing an amino acid causes a varied reaction. Most of the amino acids seem to nullify the effect of lead since there is no significant difference between the amount of growth whether lead is present or not. Some amino acids - threonine, methionine, serine, cysteine and tryptophan - produced a reaction similar to the medium without an amino acid, i.e. there was significantly less growth when lead was added. These amino acids are unable to nullify the effect of lead fully, but possibly reduce it slightly. Four amino acids produced a significant increase in growth in the presence of lead. They are ∝ aminobutyric acid, norleucine, tyrosine and arginine.

One reason for the increased growth in the presence of lead and for the many showing no significant difference may be the different mode of growth in the presence of lead. In all the different solutions containing lead there is a reduction in sporulation when compared with the colonies when lead is absent. The amount of reduction varies, with some colonies producing no visible sign of sporulation while others visibly show signs of sporulation but not to the extent of the controls. This may give rise to an increase in mycelium production since no energy is diverted to spore production. Mycelium production may therefore give rise to this increase in the presence of lead. Despite this some solutions do not give rise to more growth in the presence of lead so that there are probably other factors involved.

Heavy metals are known to react with amino acids forming strongly bound complexes so that interaction in the media might seem likely to affect the reaction of A. fumigatus to lead plus amino acids.

However, many metal complexes break down under acid conditions. Zlochevskaya and Rabotnova (1968) did not find any formation of lead complexes with amino acids in acid conditions, with the exception of cysteine. Consequently it was assumed unlikely that any complexes would be formed in these solutions at pH 3 which would interfere with the effect of lead on the growth of A. fumigatus in the experiment. The complex formed with cysteine was found to be toxic to Aspergillus niger (Zlochevskaya & Rabotnova 1966, 1968) and would seem to act similarly on A. fumigatus.

Heavy metals are generally considered to act mainly at the cell wall and there is only some penetration in to the cell. It would seem likely that the action of amino acids in allowing similar or increased growth in the presence of lead may also be sited at the cell wall. The amino acids may compete for or alter the active sites to which lead may become attached and therefore prevent the lead from acting so efficiently. Some of the variation in the reaction may be due to relative competitive ability of the amino acids in this situation.

Some amino acid activity may be involved with the lead which penetrates into the cell. If a metabolic pathway requires a particular amino acid in order to build a necessary constituent of the cell then it is possible that lead could block that pathway. If, however, the amino acid is present in the culture solution then the necessity for this synthetic ability is no longer needed. The fungus may be able to utilise an alternative pathway if another amino acid is present, so by-passing the effect of lead. This may lead to the fungus utilising amino acids it may not normally use.

Those amino acids producing increased growth would seem to be interesting in view of the stimulus as well as those which produce

no alteration in the amount of growth in the presence of lead. However, it is difficult to suggest possible mechanisms of action of amino acids from an observational experiment and a further investigation is necessary to clarify the way amino acids act.

5.9 Comparison of the growth of several isolates of Aspergillus fumigatus Fres. in the presence of lead

It was decided to compare the growth in the presence of lead of isolates of A. fumigatus from different sources to see whether the isolate from the spoil heap was better able to grow in contact with lead. The other isolates were obtained from C.M.I. and had been isolated from varied substrates.

5.9.1 Experimental Procedure

The flasks were set up normally with some of them containing lead at concentrations of 110 and 160 p.p.m. Some control flasks were also used. The flasks were harvested after 20 days growth at 25°C.

5.9.2 Results and Discussion

The results are presented in Table 84 and some in Fig. 5.10.

The results have been analysed using the analysis of variance on the results for the different lead concentrations and the control medium. In all types of medium there is significant variation among the isolates. In the control and 160 p.p.m. solutions the variance is very high being significant at the 1% level, while in 110 p.p.m., the variance is less being significant only at the 5% level. Although there is much variation in the control medium it is not thought that Czapek Dox is a selective medium, but there may be some slight variation in the ability of the isolates to utilise it as well as inherent variation between the isolates.

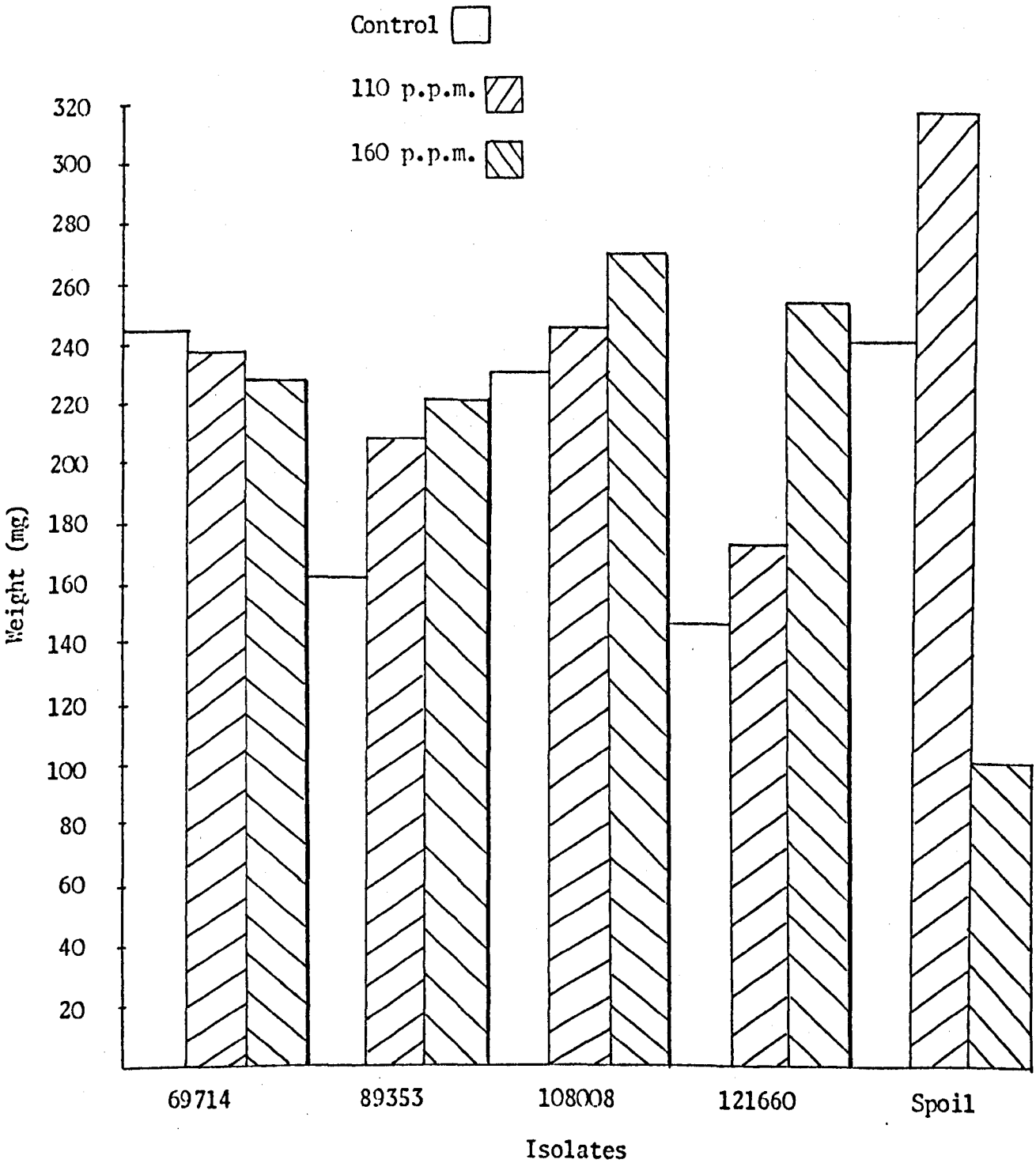


Figure 5.10

Growth of some Isolates of A. fumigatus in lead-free solutions and solutions containing 110 and 160 p.p.m. lead

The spoil isolate grew well in the medium containing 110 p.p.m. lead. However, four isolates - 96202, 16062, 28646, 45338 - did not differ significantly from the spoil isolate in their amount of growth.

In a comparison between the growth of the isolates at this concentration and their growth in control solutions there is generally an increase in weight in the solution containing the lead. This increase in the amount of growth of most isolates is probably due to the different type of growth which occurs in the two solutions. In the control flasks the cultures are heavily sporing whereas in the lead solutions the growth is mainly mycelial with little spore production. The dry weight of mycelium is greater than that of spores so that there would be an increase in weight which may not reflect a true increase in growth but a variation in the type of growth. There would be no diversion in lead solutions of energy to spore production and all the energy would be directed to mycelial extension.

Growth in the solutions containing 160 p.p.m. lead is distinctly less than in the control solutions in most isolates. A few seem unaffected by lead concentration and others show slightly more growth and may be a consequence of the different type of growth described above. The isolate from spoil does not grow well at this 160 p.p.m. concentration and does not seem to have any advantage over the other isolates. There are four isolates which do not differ from the spoil isolate and they are - 35570, 28646, 89354, 45338, two of these were similar in the other concentration. However, most of the isolates differ significantly from the spoil isolate in growing more. There are four isolates which show very markedly more growth than the spoil isolate. Two of these - 69714 and 89353 - do not show much alteration in their amount of growth in all the solutions, although there is

a slight increase in growth by the latter isolate. The reasons for the apparent lack of effect of the lead is unknown and a further investigation would seem to be warranted. Two other isolates which would seem to merit further study are 108008 and 121660 which show good growth in the 160 p.p.m. solutions. These are the only two isolates which do not produce much colour in their colonies and it is possible that some final steps in a biochemical chain cannot be completed. If the intermediate substance, which would accumulate under these conditions, were able to combine with lead or even if the enzyme combined with lead this would be an effective method of decreasing lead toxicity, as it would be removed from the cell metabolism.

The isolate from the spoil heap is a very fast spreading fungus which grows very thinly over agar media producing a reasonable amount of spores, whereas these other isolates are all strong growers producing thick colonies and in some cases a very large number of spores. This is the type of growth exhibited by isolates 69714 and 89353 and the reason for their tolerance of lead in comparison with the spoil isolate is not known. An investigation of the differences between these four isolates and the spoil isolate would be necessary to try and understand their varied reaction to lead, if it is based on differences in their metabolism or mode of growth.

There is no straightforward relationship between the different isolates and their reaction to lead in the medium. It would seem that the isolate from the spoil heap has no marked ability to tolerate lead at high concentrations to a greater degree than the other isolates. In fact some of the other isolates can tolerate lead more efficiently than the spoil isolate. The reason for this is not known. It may be due to the different type of growth of the various isolates, although

one of the more tolerant ones grew similarly to the spoil isolate. It may be that the spoil isolate is more suited to the hostile conditions in the field than those which prevail in the culture solution, and this is a difficult hypothesis to substantiate except by saying that it was the form which was isolated from there.

5.10 A comparison of the spore production of some isolates of Aspergillus fumigatus Fres. grown in different lead concentrations

As the previous experiment seemed to indicate some differences in spore production by various isolates in different lead solutions and it was decided to examine this point.

5.10.1 Experimental Procedure

Replicate flasks were set up, inoculated and left to grow for 20 days. Some of the flasks contained lead to concentrations of 110 and 160 p.p.m. and others were control solutions with no lead. The amount of sporulation was measured as described in Chapter 1.

5.10.2 Results and Discussion

The results are presented in the following table.

Table 5.3

Spores/ml of culture solution of several A. fumigatus isolates after 20 days of growth

	Number of spores x 10^4		
Isolate	Control	110 p.p.m. Pb	160 p.p.m. Pb
Spoil	1053	219	70
89353	18	15	3
108008	2	2	1
121660	36	10	0.3
96202	1440	525	222
69714	1125	500	135
45338	333	147	2

All the isolates which were tested decreased their spore production as the concentration of lead increased. The amount of decrease varied as some isolates only produced a small number of spores initially. In this respect isolate 108008 hardly varies with the lead content as is to be expected for its numbers are very low. Nearly all the isolates which grew more than the spoil isolate in high lead solutions produced markedly fewer spores suggesting that they are strong mycelial forms. There were two, however, (69714 and 96202) whose spore production were not so obviously affected as the others and this may reflect some attribute which makes them more able to tolerate lead. It would seem, therefore, that spore production is not a simple indicator of the tolerance of various isolates to lead. This is confirmed by isolate 45338 which resembled the spoil isolate in 160 p.p.m. but produced fewer spores, an attribute in the other isolates had been associated with increased tolerance. However, there does seem to be some relationship between spore production and tolerance since the dubious isolates may be due to inherent variation between isolates. The fact that spore production and lead tolerance are not obviously connected does not mean to say that there is no relationship between them since the exceptions to this rule may be due to some other form of variation.

The spoil isolate is possibly adapted to produce many spores from a sparse mycelium and can therefore grow and spread in the soil from which it was isolated. It may be unable to readapt to the conditions found in the liquid medium and so it may be at a disadvantage in the experiment if the other isolates are used to a richer substrate. Phenotypic variation may, of course, be the reason for the difference in the results but it is not likely to be the main cause of such marked variation. This could be tested using single spore isolates

and growing the colonies produced in experimental solutions and examining the variation. It would seem more likely that there are genetic differences of metabolism in the various isolates which gives rise to their varied ability to tolerate lead.

5.11 Comparison of the growth of two isolates of several fungal species in the presence of lead

It was decided to compare isolates of various fungi from the spoil heap with isolates of the same fungi from some other site in order to see if there was any obvious adaptation to lead on the part of the spoil heap isolates.

5.11.1 Experimental procedure

Replicate flasks were set up with Czapek Dox medium, some containing 160 p.p.m. lead. Isolates of the species from the spoil and others of the same species from C.M.I. were used, and one isolate from C.B.I. The flasks were inoculated using a sterile inoculating needle since not all of the cultures gave rise to suitable spore suspensions. Variation in the inoculum may have affected the results slightly. The species used were listed in Chapter 1.

5.11.2 Results and Discussion

The results are presented in Table 85 and Fig. 5.11

The different species showed a varied reaction in their ability to grow in the control solutions which is probably a reflection of the difference in their ability to utilise the nutrients in the medium. This may affect their ability to withstand the action of lead since some may be better suited to the conditions in the flask and so their growth may be stronger.

Growth of the isolates from spoil in 160 p.p.m. showed some

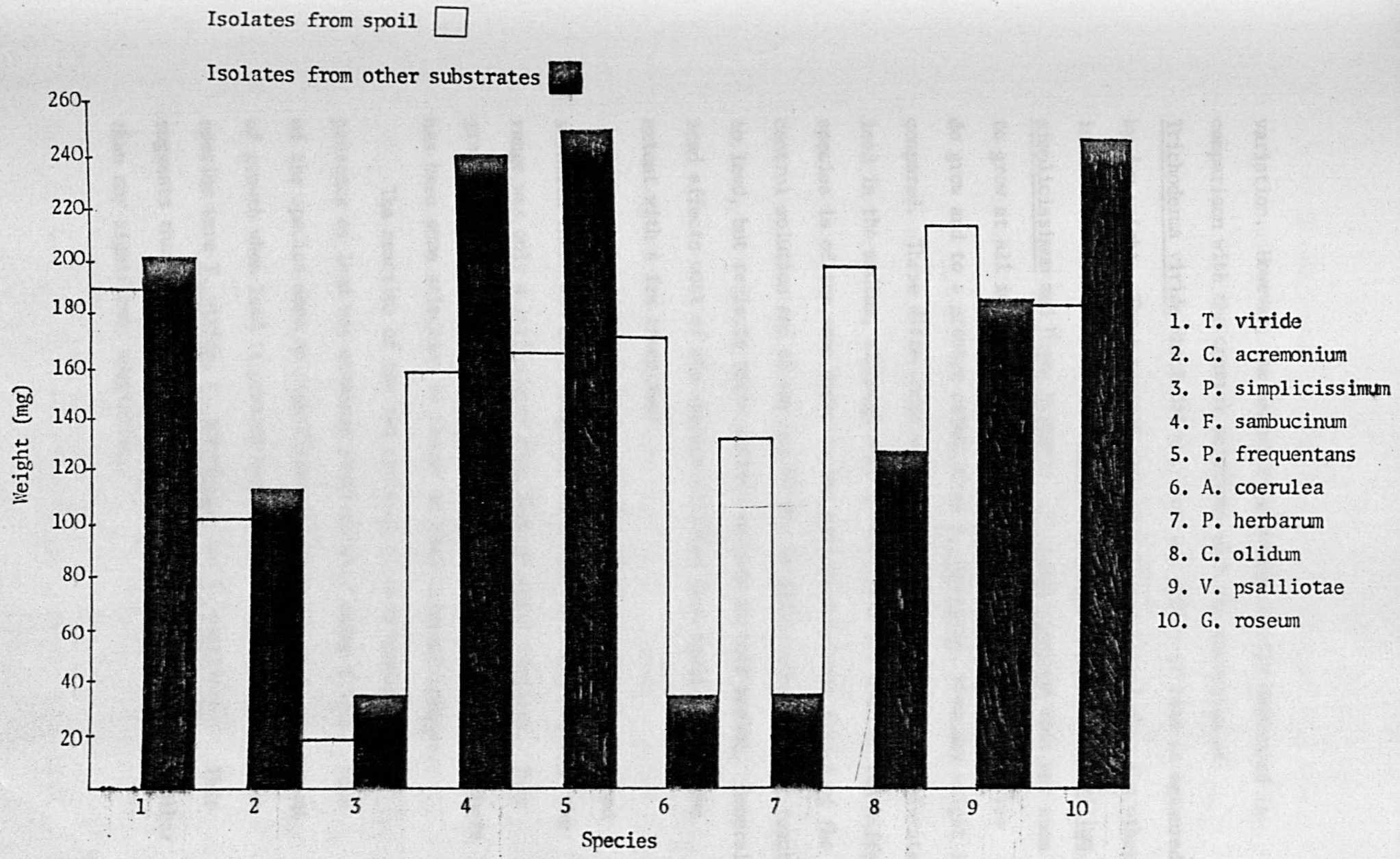


Figure 5.11

Growth of Isolates from different substrates of several species in 160 p.p.m. lead

variation. However, the amount of growth generally decreased in comparison with the control solutions with the exception of Trichoderma viride which did not show any effect of lead as measured by dry weight. Three species grew to a lesser extent than the others in the lead solution, namely Cephalosporium acremonium, Penicillium simplicissimum and Phoma herbarum. P. simplicissimum does not seem to grow at all in the presence of lead, but the two other species do grow and to a greater extent than A. fumigatus, when dry weight is compared. These differences may reflect varying ability to tolerate lead in the medium, although some of the variation between the different species is of the same order as the variation between flasks of the control solution and so may not be due to differences in their reaction to lead, but reflects their ability to grow in this medium. Generally lead affects most of the species isolated from spoil to the same extent with a few exceptions.

When the isolates from other substrates were grown in lead solution there was much variation in their reaction, although the range was only a little more than that of spoil isolates. This greater variety may indicate that amongst the spoil isolates there has been some selection in favour of lead tolerant species.

The reaction of the two isolates of each species in the presence of lead was examined statistically using t test. Some of the species show no significant difference between the amount of growth when lead is present between the two isolates. These species were T. viride, C. acremonium and V. psalliotae. This suggests that these species have an inherent lead tolerance rather than any significant adaptation.

In some species the isolates from other substrates show a significant increase in their growth in the presence of lead when compared with the spoil isolates. These were F. sambucinum var. caeruleum, P. frequentans, G. roseum and P. simplicissimum with varying levels of significance. P. simplicissimum although significantly different in fact grows extremely sparingly and may not grow to any significant amount. These species show no evidence of any specific adaptation.

A. coerulea, P. herbarum and C. olidum are particularly interesting. The isolates from spoil of these species grew significantly more in the presence of lead than the isolates from other substrates. This may indicate some adaptation by these species to the lead contaminated soils. This reaction is interesting with regard to P. herbarum which is commonly isolated from spoil heaps. Isolates of Phoma have also been known to attack lead paint (Eveleigh 1961) so that it would seem to be a fungus which can adapt or tolerate lead.

This experiment indicates that there are two components of the fungal flora of the spoil heaps containing lead. One is a group of fungal species whose metabolism is such that even under normal lead-less circumstances they are highly tolerant of lead in the environment. The other group consists of species which are tolerant of lead normally but which have produced strains in the spoil heap which have a much higher lead tolerance level. This latter group might indicate some adaptation similar to that found in grasses (Jowett 1958, 1964).

5.12 Growth of some species in a higher concentration of lead

Since some of the species which were examined seemed to grow well in 160 p.p.m. it was decided to see if they could tolerate a

higher concentration of lead.

5.12.1 Experimental Procedure

Replicate flasks were set up containing 200 p.p.m. lead. They were harvested after 20 days. They were inoculated using an inoculating needle.

5.12.2 Results and Discussion

The results are presented in Table 86 and Fig. 5.12.

Most of the species showed little if any growth at this concentration, so that the level of tolerance is somewhere between 200 and 160 p.p.m. lead. It is noteworthy that the A. fumigatus from the spoil heap grew better at 200 p.p.m. lead than any of the other A. fumigatus isolates. This might be considered surprising since many of these isolates grew better at 160 p.p.m. of lead than the spoil isolate. The conclusion one must draw therefore is that the isolate from spoil, although it does not grow so well as the other isolates at low concentrations, is more tolerant of lead over a wider range which would undoubtedly put it at an ecological advantage in a spoil heap where there are local concentrations of the metal.

Fusarium sambucinum var. caeruleum and Cephalosporium acremonium grow slightly more than A. fumigatus from spoil but it does not seem a significant amount. In fact the total amount of growth is very small and probably not very significant, possibly being due to variation of filtering rather than any active growth. However, two species grew quite considerably at 200 p.p.m. and they were Verticillium psalliotae and Cylindrocarpon olidum. V. psalliotae is very tolerant of lead and grows in a thick mat on the surface of the medium. The amount of mycelium in contact with lead is very

1. A. fumigatus - 69714
2. A. fumigatus - 89353
3. A. fumigatus - 108008
4. A. fumigatus - 121660
5. V. psalliotae
6. C. olidum
7. A. coerulea
8. F. sambucinum
9. C. acremonium
10. A. fumigatus from spoil

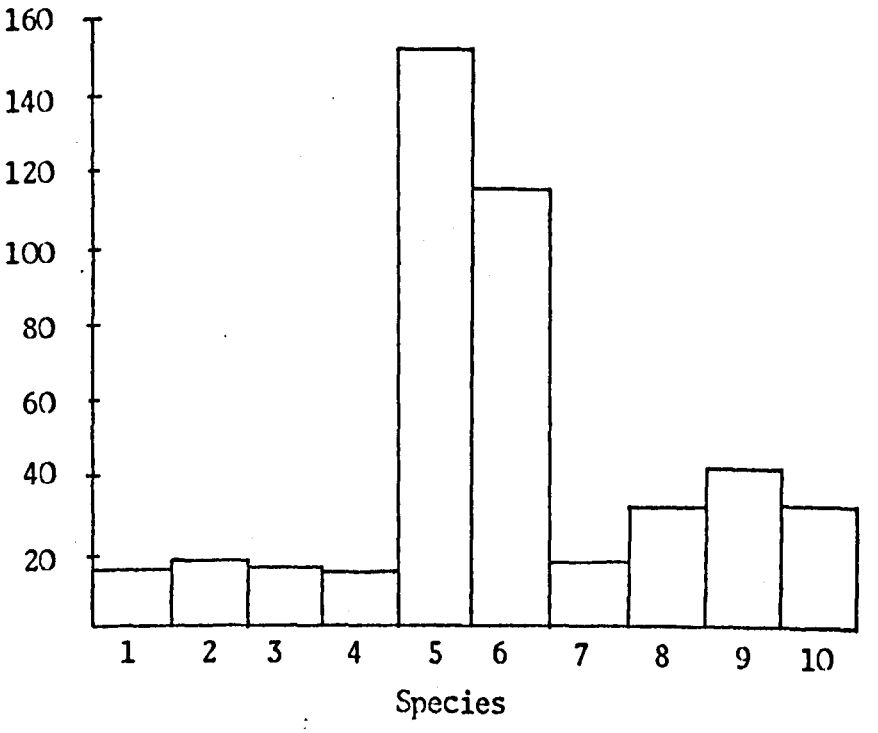


Figure 5.12

Growth of some Species in 200 p.p.m. lead

small so that only some mycelial growth may be affected. C. olidum in the medium produces a slimy mycelium with very little spore production. This is different from the normal growth of the fungus and clearly reflects some physiological difference due to the presence of lead in the medium.

5.13 Growth of several species in prolonged culture in 100 p.p.m. lead

It was decided to examine whether prolonged growth in lead contaminated media gave rise to an increase in the amount of growth as was shown for some fungicides by Partridge and Rich (1962). This might indicate a gradual adaptation of the fungus or merely selection of the stronger growing strains from within a mixed inoculum.

5.13.1 Experimental Procedure

Four flasks were set up for each isolate containing 100 p.p.m. lead. After 20 days the medium in three of the flasks was filtered and the dry weight established. The fourth flask with growth of the isolate was used to inoculate four fresh flasks of medium with 100 p.p.m. lead. This procedure was carried out eight times to that the fungus was in contact with lead for 180 days. The fungi used in this experiment were Aspergillus fumigatus from spoil and isolates 108008 and 121660, Verticillium psalliotae and Fusarium sambucinum var. caeruleum.

5.13.2 Results and Discussion

The results are presented in Table 87 and Figs. 5.13 and 5.14.

There was much variation between the amounts of growth at each transfer, some of which may be a reflection of the variation between the inoculum since pieces of mycelium from the flasks are used.

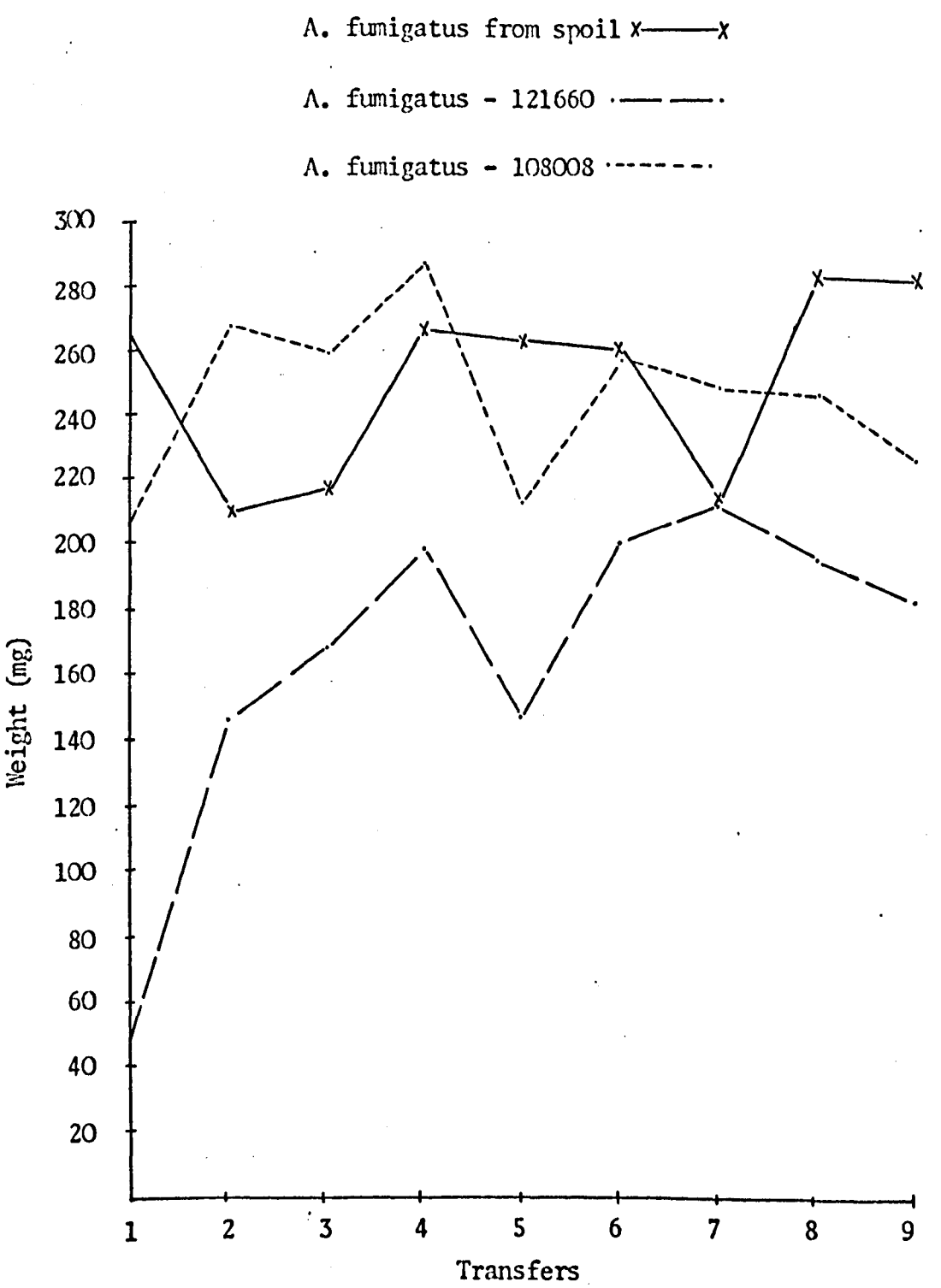


Figure 5.13

Prolonged growth of three Isolates of A. fumigatus in media containing 100 p.p.m. lead

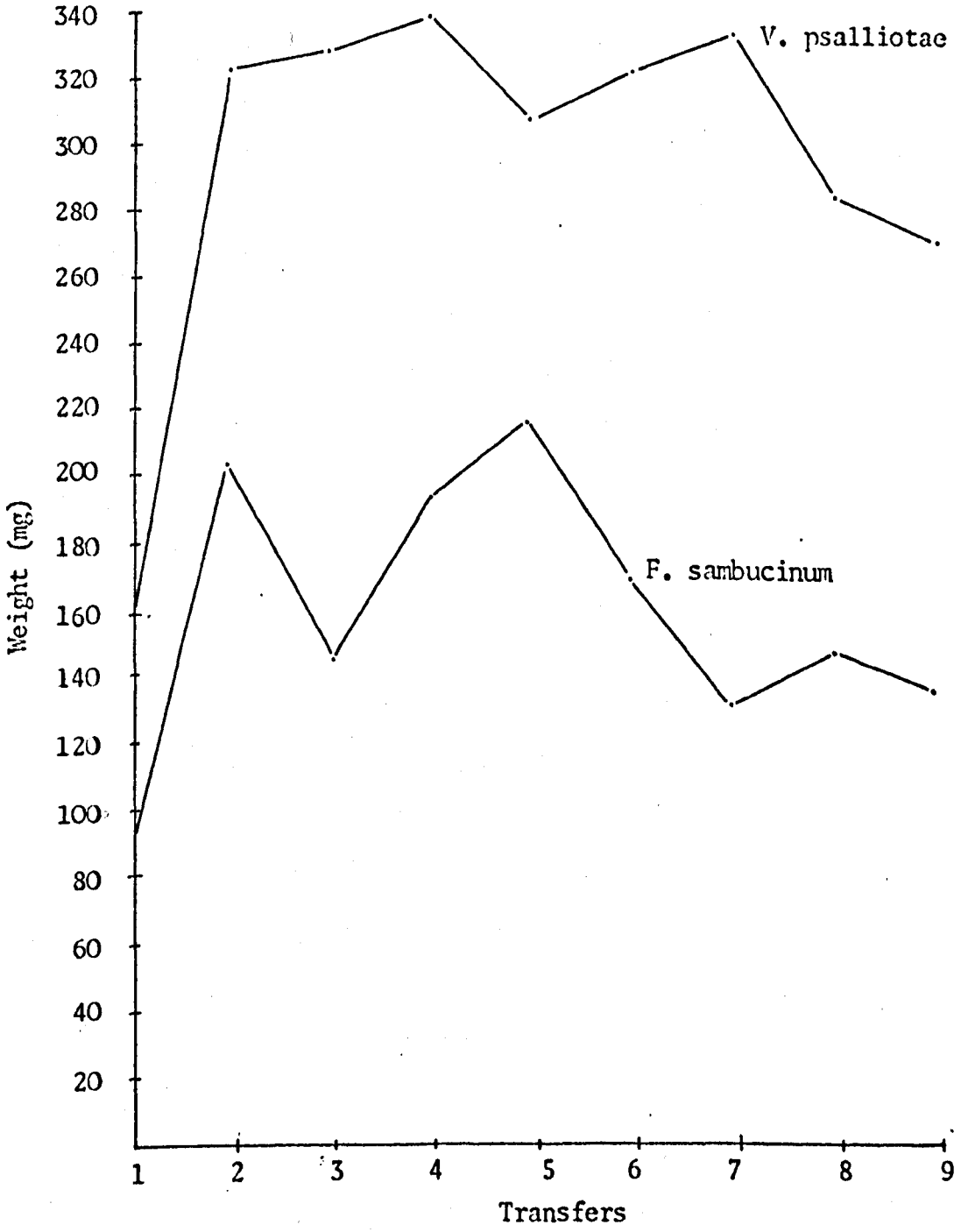


Figure 5.14

Prolonged growth of F. sambucinum var. caeruleum and V. psalliotae in media containing 100 p.p.m. lead

Although the amount of growth of A. fumigatus from spoil fluctuates generally the same level of growth is maintained so that there is seemingly no adaptation or selection as reflected by increased growth. Similarly isolate 108008 shows some variation between transfers but the general pattern is the same. However, 121660 increases its growth in the first few transfers and then the same amount is maintained. This might indicate some form of adaptation or selection taking place so that the fungus is able to grow to a greater extent. It would appear to be permanent once the change has taken place since growth was maintained at a higher rate once it was achieved, so that it may be some genetical change.

After an initial rise V. psalliotae remains steady in its amount of growth and then begins to decline slightly. The initial rise may be due to some form of adaptation to the conditions in the medium or possibly due to selection of the stronger growing parts. The slight decline may be due to some loss of viability of the culture. Fusarium sambucinum var. caeruleum varies to a greater extent than A. fumigatus but also shows little overall change indicating any adaptation to the medium.

These results indicate that some fungi can adapt to lead resulting in increased growth or that different parts of the colony can grow better in the presence of lead and so contribute more to later transfers so that there is selection. Further study would be necessary to clarify the different reactions of the fungi and the basis of these reactions.

5.14 Summary Discussion

Since many lead salts are insoluble the use of lead for experimentation gives rise to problems of uniform distribution

in a liquid or colloidal environment and the possibility of lead depriving the fungus of some necessary nutrient such as phosphate. In a comparison of the growth of Aspergillus fumigatus on lead-containing solid and liquid media there were discrepencies in growth which may be a consequence of the differences in surface area of the fungus which is in contact with the lead. Although growth is less in liquid medium containing lead, conditions are more homogeneous and so liquid culture was used for most experiments. However, to ensure the uniform availability of nutrients and lead it was necessary to acidify the medium to pH 3. This pH did not affect the growth of A. fumigatus or the other fungi to any marked degree. In liquid experiments at pH 3 it was found that growth was little affected by lead up to a concentration of 120 p.p.m. lead and then the amount of growth decreased as the lead content of the medium increased until at 180 p.p.m. there was no growth as measured by dry weight.

Lead delayed the germination of A. fumigatus spores although the eventual percentage of spores which germinated even up to a concentration of 250 p.p.m. lead was similar to that in a lead-free medium. The presence of lead in the hanging drop gave rise to a dependence on external nutrients before germination would occur. The reduction in germination brought about by lead was in reality simply a delay in the emergence of the germ tube and this delay may manifest itself in another form as there is clear indication that as the proportion of lead in the medium increased so the growth of the germ tube decreased. These two effects namely the apparent delay in germination and slowing down of growth in the presence of lead may be different facets of the same phenomenon.

The stage of growth at which lead takes effect was investigated

by growing A. fumigatus in a lead-free solution and then transferring the growth after different periods of time to a medium containing 170 p.p.m. lead. Lead clearly affected the early growth of the fungus since the longer the time allowed for growth in a medium without lead then the less effect lead had on subsequent growth in a lead-containing medium.

After 6 days in a lead-free medium the growth curve following transfer to a medium containing 170 p.p.m. lead is similar to the growth of a fungus in a lead-free medium throughout, showing no period of acclimatisation. Initial growth of A. fumigatus in a medium containing 170 p.p.m. lead showed no adverse effect on transference to a lead-free medium and it can be tentatively concluded that lead slows the metabolism without effectively destroying any part of it. It is possible, for example, that the presence of lead prevents certain biochemical pathways from being used and the alternative pathways, which clearly must exist since the fungus grows, only operate more slowly or with significantly less efficiency. It was thought that supplementation of the medium with amino acids might help the fungus to eliminate the slowing effect of lead. There was some evidence that growth was improved but not to any marked extent by all amino acids tested.

In addition to the growth experiments it was decided to investigate experimentally the ecology of lead tolerant fungi by comparing the lead tolerance of isolates from spoil with those from other substrates. There was much variation between the different isolates of A. fumigatus. In low concentrations of lead (110 p.p.m.) the isolate from the spoil grew best, although most of the isolates showed more growth than in lead-free media. But in a higher concentration (160 p.p.m.) the spoil isolate grew weakly by comparison

and some of the other isolates grew very well showing high tolerance. Since the spoil is not a uniform but rather a very variable substratum the amount of lead in any particular locality may differ from that in a nearby area. The competitive power of the isolate of A. fumigatus from spoil probably resides in its tolerance of a wide range of lead concentrations rather than in its ability to grow well at any given concentration. Some of the other isolates might do well locally, but would not be able to colonise the whole spoil heap.

Amongst the other species of fungi and their isolates from lead and lead-free situations there was a mixed reaction to the presence of lead. Some of the species were normally lead tolerant and showed no change in growth in the presence of lead while other species were susceptible to lead as evidenced by a decreased growth rate. It would seem therefore that some strains of fungi have a built-in tolerance of lead while others do not, although the latter may develop a tolerance which enables them to live in the spoil. Successive transfers of a number of species in 100 p.p.m. lead-containing medium showed little evidence of any selection of particular genotypes from within what was probably a mixed population. There may have been some immediate selection but there was little build-up after the first two transfers.

The difficulties and the time-consuming nature of these experiments has meant that the results are suggestive rather than conclusive. However, they do point to the path along which future investigators should travel when examining the effect of lead on Aspergillus fumigatus and other fungi.

SECTION III

APPENDIX

TABLE 1

List of different species isolated from each soil sample from Site 1

Sample 1	Aspergillus fumigatus
	Aspergillus glaucus
	Chaetomium indicum
	Cladosporium herbarum
	Coniothyrium fuckelii
	Penicillium cyclopium
	Penicillium funiculosum
	Penicillium tardum
	Sterile mycelia
Sample 2	Cephalosporium acremonium
	Mucor hiemalis
	Rhizopus arrhizus
Sample 3	Chaetomium homopilatum
	Penicillium citrinum
	Phoma herbarum
Sample 4	Absidia coerulea
	Aspergillus terreus
	Epicoccum purpurascens
Sample 5	Penicillium frequentans
	Scopulariopsis brevicaulis

TABLE 2

List of different species isolated from each soil sample from Site 2

Sample 1	Chaetomium homopilatum
	Coniothyrium fuckelii
	Penicillium funiculosum
	Phoma herbarum
	Sterile mycelia
Sample 2	Aspergillus fumigatus
Sample 3	Cephalosporium acremonium
	Cylindrocarpon olidum
	Gliocladium roseum
	Penicillium corymbiferum
	Penicillium notatum
	Trichoderma viride
	Yeast species
Sample 4	Alternaria tenuis
	Aureobasidium pullulans
Sample 5	Mucor hiemalis
	Rhizopus arrhizus
Sample 6	Cephalosporium roseo-griseum
	Sporotrichum chlorinum
Sample 7	Botrytis cinerea
	Fusarium solani
	Verticillium candelabrum
Sample 8	Absidia coerulea
	Penicillium canescens

TABLE 3

List of different species isolated from each sample from Site 3

Sample 1	Absidia butleri
	Absidia coerulea
	Aspergillus fumigatus
	Penicillium funiculosum
	Phoma herbarum
	Sterile mycelia
Sample 2	Cephalosporium acremonium
	Cephalosporium roseo-griseum
	Cylindrocarpon olidum
	Penicillium tardum
	Rhizopus arrhizus
Sample 3	Coniothyrium fuckelii

TABLE 4

List of different species isolated from each soil sample from Site 4

Sample 1	Aspergillus glaucus
	Aspergillus fumigatus
	Chaetomium homopilatum
	Eladia saccula
	Mucor hiemalis
	Penicillium brevi-compactum
	Penicillium funiculosum
	Penicillium luteum
	Penicillium nigricans
	Penicillium ochro-chloron
	Penicillium simplicissimum
	Penicillium waksmani
	Sterile mycelium
	Trichoderma viride
Sample 2	Fusarium culmorum
	Penicillium lilacinum
	Phoma herbarum
Sample 3	Coniothyrium fuckelii
	Gliocladium roseum
Sample 4	Botrytis cinerea
	Penicillium notatum

TABLE 5

List of different species isolated from each sample from Site 5

Sample 1	Absidia spinosa
	Chaetomium indicum
	Fusarium solani
	Mucor hiemalis
	Penicillium funiculosum
	Phoma herbarum
	Trichoderma viride
Sample 2	Alternaria tenuis
	Fusarium sambucinum var. caeruleum
	Penicillium aculeatum
	Penicillium brevi-compactum
	Penicillium notatum
	Penicillium simplicissimum
	Sterile mycelia
Sample 3	Cladosporium herbarum
	Penicillium cyclopium
	Penicillium palitans
Sample 4	Absidia repens
	Aspergillus glaucus
	Penicillium lilacinum
	Penicillium waksmani
	Verticillium candelabrum

TABLE 5

List of different species isolated from each sample from Site 5 (Continued)

Sample 5	Absidia coerulea
	Penicillium nigricans
Sample 6	Botrytis cinerea
	Epicoccum purpurascens
Sample 7	Mucor corticolus
	Mucor varians
Sample 8	Aureobasidium pullulans
	Paecilomyces varioti
	Penicillium rubrum
Sample 9	Cephalosporium acremonium
	Gliocladium roseum
	Rhizopus oryzae

Table 6

Number of different Species occurring on each soil plate from Site 1

	Plates			
Samples	1	2	3	4
1	4	9	9	9
2	10	12	12	12
3	15	15	15	15
4	16	18	18	18
5	19	20	20	20

Table 7

Number of different Species occurring on each soil plate from Site 2

	Plates			
Samples	1	2	3	4
1	1	4	4	5
2	6	6	6	6
3	9	11	12	13
4	14	14	14	15
5	17	17	17	17
6	17	18	18	19
7	20	21	21	22
8	24	24	24	24
9	24	24	24	24
10	24	24	24	24

Table 8

Number of different Species occurring on each soil plate from Site 3

	Plates			
Samples	1	2	3	4
1	3	5	5	6
2	9	11	11	11
3	11	12	12	12
4	12	12	12	12
5	12	12	12	12

Table 9

Number of different Species occurring on each soil plate from Site 4

	Plates			
Sample	1	2	3	4
1	10	13	13	14
2	17	17	17	17
3	19	19	19	19
4	20	20	20	21
5	21	21	21	21

Table 10

Number of different Species occurring on each soil plate from Site 5

	Plates			
Sample	1	2	3	4
1	3	5	6	7
2	7	9	13	14
3	14	16	17	17
4	17	20	22	22
5	22	23	24	24
6	24	26	26	26
7	26	27	28	28
8	28	30	31	31
9	31	31	33	34
10	34	34	34	34

TABLE 11

Site 1 - Assay for May 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus amstelodami							25	12.5
Aspergillus fumigatus	50	27.7	75	66.6	25	5		
Cephalosporium asperum							25	12.5
Cephalosporium curtipes			25	8.3				
Chaetomium globosum					25	10		
Coniothyrium fuckelii					25	10		
Emericellopsis species					25	10	50	50
Fusarium species					25	5		
Mortierella jenkini					25	5		
Mucor species					25	10		
Nodulisporium species	25	5.5						
Penicillium frequentans					25	15	25	12.5
Penicillium funiculosum					25	5		
Penicillium waksmani	25	5.5						
Phoma herbarum	50	27.7	25	16.6			25	12.5
Sordaria fimicola					25	5		
Sporotrichum chlorinum	25	5.5						
Sterile Mycelia	25	11.1	25	8.3	50	20		
Trichoderma viride	50	16.6						
Total Isolates	18		12		20		8	
Total Species	7		4		11		5	

TABLE 12

Site 1 - Assay for July 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	12.5	75	52.9	50	25		
Candida species	25	12.5						
Chaetomium funicola	25	12.5						
Epicoccum purpurascens			25	5.8				
Oospora variabilis			25	5.8				
Penicillium cyaneo-fulvum					25	12.5		
Penicillium funiculosum	25	12.5						
Penicillium jenseni			25	5.8				
Penicillium velutinum	25	12.5						
Phoma herbarum					25	12.5		
Phoma violacea	25	12.5	25	23.5	50	25	25	100
Sterile Mycelia	50	25	25	5.8	50	25		
Total Isolates	8		17		8		1	
Total Species	7		6		5		1	

TABLE 13

Site 1 - Assay for September 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	5.5	25	16.6	50	36.8		
Absidia glauca			25	16.6				
Alternaria humicola	25	5.5						
Aspergillus amstelodami	25	5.5						
Aspergillus fumigatus	50	16.6	25	16.6	75	21		
Aspergillus versicolor	25	5.5	25	33.3	25	15.7		
Candida species	50	22.2						
Coniothyrium fuckelii	25	5.5						
Geotrichum species	25	5.5			25	5.2	25	100
Penicillium declauxi					25	5.2		
Penicillium fellutanum					25	5.2		
Penicillium notatum	25	5.5						
Phoma herbarum	50	11.1			25	5.2		
Pyrenochaeta decipiens	25	5.5						
Sterile Mycelia	25	5.5	25	16.6	25	5.2		
Total Isolates	18		6		19		1	
Total Species	12		5		8		1	

TABLE 14

Site 1 - Assay for November 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria humicola	25	9.3						
Aspergillus fumigatus			25	6.2	25	4		
Aspergillus versicolor	25	3.1						
Arthrinium phaeospermum			25	12.5				
Aureobasidium pullulans			25	6.2				
Candida species					25	8		
Cephalosporium acremonium					25	8		
Cylindrocarpon olidum					25	4		
Epicoccum purpurascens	50	9.3						
Fusarium culmorum			25	6.2			25	9
Fusarium oxysporum					25	4		
Fusarium sambucinum var. caeruleum	50	6.2						
Gliocladium roseum					50	8		
Oospora sulphurea			25	6.2				
Penicillium brevi-compactum	25	6.2			25	4		
Penicillium cyclopium	25	3.1	25	6.2	25	8	25	18.1
Penicillium funiculosum	25	3.1	25	12.5				
Penicillium lanosum	25	3.1			25	4		
Penicillium notatum					25	4	25	9
Pestalotia pezizoides					25	4		
Phoma glomerata	50	15.6						
Phoma herbarum	50	6.2	25	6.2	25	4		
Phoma species	50	9.3	25	6.2	75	24		
Sterile Mycelia	50	9.3	75	25	25	8	100	63.6
Trichoderma viride	50	15.6	25	6.2	25	4		
Total Isolates	32		16		25		11	
Total Species	13		11		15		4	

TABLE 15

Site 1 - Assay for January 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	75	15.3	50	9.5	25	50		
Cephalosporium acremonium	25	3.8						
Chaetomium homopilatum			50	9.5				
Chaetomium indicum			25	4.7				
Coniothyrium fuckelii					25	50		
Mucor hiemalis	25	3.8						
Mucor microsporus	25	3.8	25	9.5				
Mucor spinescens	25	7.6	25	19			25	50
Penicillium brevi-compactum			25	4.7				
Penicillium cyclopium	25	7.6						
Penicillium decumbens			50	9.5				
Penicillium frequentans	25	3.8	50	9.5				
Penicillium funiculosum			25	4.7				
Penicillium lilacinum	50	11.5	25	4.7				
Penicillium notatum	25	3.8						
Penicillium ochraceum	25	3.8						
Penicillium simplicissimum	25	7.6						
Penicillium urticae	25	3.8						
Phoma herbarum	25	3.8						
Sterile Mycelia	25	11.5	25	9.5				
Trichoderma viride	25	7.6	25	4.7				
Verticillium cinnabarina							25	50
Total Isolates	26		21		2		2	
Total Species	15		12		2		2	

TABLE 16

Site 1 - Assay for March 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus chevalieri var. intermedius	25	25						
Sterile Mycelia	25	75						
Total Isolates	4							
Total Species	2							

TABLE 17

Site 1 - Assay for May 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria humicola	25	4						
Aspergillus chevalieri	25	4					25	28.5
Aspergillus versicolor							25	14.2
Aureobasidium pullulans	25	4						
Epicoccum purpurascens	25	4						
Geotrichum candidum	25	4						
Mucor microsporus	25	12						
Mucor varians	25	4						
Paecilomyces species			50	100				
Penicillium cyclopium	50	12						
Penicillium funiculosum							25	14.2
Penicillium spinulosum	50	8						
Phoma herbarum	50	28			25	50	25	28.5
Sterile Mycelia	25	12			25	50	25	14.2
Yeast species	25	4						
Total Isolates	25		3		2		7	
Total Species	12		1		2		5	

TABLE 18

Site 1 - Assay for July 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia heterospora	25	4.1						
Absidia spinosa	25	4.1						
Aspergillus aureolus	50	8.3	25	5.8				
Aspergillus fumigatus	50	12.5					25	9
Botrytis cinerea	25	12.5			25	14.2		
Candida albicans	25	4.1						
Cephalosporium curtipes			25	5.8				
Cladosporium cladosporioides					50	21.4		
Cladosporium herbarum	50	8.3	25	5.8			25	9
Coniothyrium fuckelii			25	11.7	25	7.1		
Epicoccum purpurascens			25	11.7				
Fusarium sambucinum var. caeruleum	25	4.1						
Penicillium funiculosum							25	9
Penicillium janthinellum			25	11.7				
Penicillium ochro-chloron	25	8.3			25	7.1	25	9
Penicillium palitans							25	9
Penicillium simplicissimum					25	7.1		
Penicillium spinulosum							25	9
Penicillium terrestre	25	4.1						
Penicillium thomii	25	4.1						
Penicillium velutinum			25	5.8	25	7.1		
Penicillium waksmani			25	5.8				
Phoma herbarum	25	4.1	25	11.7				
Sporobolomyces roseus	25	4.1					25	9
Sterile Mycelia	50	12.5	50	17.6	25	21.4	50	18.1
Stemphylium botryosum			25	5.8	25	7.1		
Trichoderma viride			25	5.8				

TABLE 18

Site 1

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Verticillium cinnabarina	25	4.1						
Verticillium psalliotae					25	7.1		
Yeast species							25	9
Total Isolates	24		17		14		11	
Total Species	15		12		9		9	

TABLE 19

Site 1 - Assay for September 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria humicola	25	2.6						
Aspergillus fumigatus	75	7.8	25	8.3				
Botrytis cinerea			25	8.3				
Cephalosporium acremonium					25	25		
Coniothyrium fuckelii	50	10.5	25	8.3				
Doratomyces stemonitis			25	8.3				
Epicoccum purpurascens	25	2.6	25	8.3				
Fusarium solani	25	2.6						
Gliocladium roseum	25	2.6						
Penicillium cyclopium	25	2.6						
Penicillium frequentans	50	5.2						
Penicillium janthinellum					25	25		
Penicillium lilacinum	25	2.6	25	8.3				
Penicillium meleagrinum	25	2.6						
Penicillium simplicissimum	25	2.6						
Penicillium thomii	25	5.2	25	8.3				
Phoma herbarum	50	10.5						
Sterile Mycelia	75	13.1	50	33.3	25	25		
Trichoderma viride	75	23.6			25	25		
Verticillium psalliotae			25	8.3				
Verticillium terrestre	25	2.6						
Total Isolates	38		12		4		0	
Total Species	16		9		4		0	

TABLE 20

Site 1 - Assay for November 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia butleri					25	66.6		
Aspergillus fumigatus	50	33.3					25	50
Botrytis cinerea	25	16.6						
Cylindrocarpon olidum	25	8.3						
Penicillium brevi-compactum			25	100				
Penicillium citrinum					25	33.3		
Penicillium funiculosum	25	8.3						
Penicillium lanosum	25	8.3						
Penicillium spinulosum	25	8.3					25	50
Sterile Mycelia	50	16.6						
Total Isolates	12		1		3		2	
Total Species	7		1		2		2	

TABLE 21

Site 1 - Assay for January 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria tenuis	25	4.1						
Aspergillus fumigatus	25	4.1	25	12.9			50	57.1
Aspergillus terreus	25	4.1						
Aspergillus versicolor	25	8.3						
Cephalosporium acremonium	25	4.1						
Chaetomium indicum			25	3.2				
Coniothyrium fuckelii	25	4.1	25	3.2				
Cylindrocarpon olidum	25	4.1						
Epicoccum purpurascens	25	4.1						
Fusarium sambucinum var. caeruleum			25	3.2				
Gliocladium roseum			25	3.2				
Mucor hiemalis					25	12.5		
Penicillium canescens			25	3.2				
Penicillium cyclopium							25	28.5
Penicillium frequentans					25	12.5		
Penicillium humuli			25	3.2				
Penicillium lilacinum			25	3.2				
Penicillium thomii			25	3.2				
Phoma glomerata	25	4.1						
Phoma herbarum	50	12.5	100	25.8				
Sterile Mycelia	50	41.6	50	35.4	50	75	25	14.2
Trichoderma viride	25	4.1						
Total Isolates	24		31		8		7	
Total Species	12		11		3		3	

TABLE 22

Site 1 - Assay for March 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	50	15.3	50	80	25	33.3	25	100
Cephalosporium curtipes	25	7.6						
Fusarium solani	25	7.6						
Mucor hiemalis					25	33.3		
Penicillium citrinum	25	7.6						
Penicillium cyaneo-fulvum	25	7.6						
Penicillium palitans	25	7.6						
Penicillium spinulosum	25	7.6						
Penicillium viridicatum	25	7.6						
Penicillium piceum	25	7.6			25	16.6		
Phoma herbarum	25	7.6						
Sterile Mycelia	50	15.2	25	20				
Trichoderma viride					25	16.6		
Total Isolates	13		5		6		2	
Total Species	11		2		4		1	

TABLE 23

Lead analysis pH and soil moisture measurements of soil samples from Site 1

Sample	Lead analysis		Top Soil		5cm		15cm		25cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
May 1970	5,400	10,560	7.0	4.8	7.4	4.9	7.7	13.0	6.4	43.0
July	7,300	14,600	8.6	2.2	8.6	3.9	8.1	28.0	8.3	25.0
September	3,300	6,400	7.6	2.3	7.1	1.6	7.4	2.9	7.2	28.0
November	12,800	17,800	6.9	7.1	6.9	7.2	6.7	32.0	6.6	10.0
January 1971	14,200	30,000	7.5	8.1	7.5	7.4	8.4	7.3	7.9	31.0
March	3,250	6,000	7.8	4.6	7.2	3.5	7.4	14.0	7.2	26.0
May	3,350	6,000	7.4	1.7	7.4	3.2	7.3	3.7	7.2	29.0
July	8,200	10,600	7.4	3.0	7.4	3.6	7.3	3.6	7.4	27.0
September	5,900	7,600	8.0	3.8	7.9	3.6	7.4	26.1	7.6	27.0
November	2,000	7,700	7.9	5.7	8.6	3.7	7.9	21.0	8.1	26.0
January 1972	11,600	13,600	7.4	1.5	7.5	2.5	7.7	1.5	7.9	13.0
March	7,900	11,600	8.2	11.5	8.1	11.5	8.1	34.0	8.1	24.0
May	12,000	13,200	7.6	0	7.6	0	7.4	27.0	7.4	24.0

TABLE 24

Site 2 - Assay for May 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria humicola							50	15.3
Arthrinium phaeospermum	25	7.1						
Aspergillus fumigatus	75	17.8	25	50	25	25	25	7.6
Aspergillus niger	25	3.5						
Botrytis cinerea	25	3.5						
Cephalosporium acremonium							25	7.6
Coniothyrium fuckelii	25	3.5						
Doratomyces stemonitis							25	7.6
Emericellopsis species	50	21.4	50	50				
Mucor hiemalis	25	3.5						
Oospora variabilis					25	25	25	15.3
Paecilomyces species	25	7.1						
Penicillium frequentans	50	10.7			25	25		
Phoma herbarum	25	3.5					25	7.6
Pyrenochaeta decipiens					25	25		
Sporotrichum chlorinum	25	3.5						
Sterile Mycelia	75	14.2					50	15.3
Verticillium cinabarina							50	23
Total Isolates	28		4		4		13	
Total Species	12		2		4		8	

TABLE 25

Site 2 - Assay for July 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	4.7	50	44.4	25	11.1		
Aspergillus fumigatus	25	2.3			25	11.1		
Aureobasidium pullulans	25	2.3						
Botrytis cinerea			25	11.1				
Cephalosporium acremonium	25	2.3						
Cephalosporium roseo-griseum	25	2.3						
Chaetomium globosum	25	2.3						
Chaetomium homopilatum	25	7.1						
Epicoccum purpurascens	25	2.3						
Penicillium brevi-compactum	25	2.3			25	11.1		
Penicillium chrysogenum					25	11.1		
Penicillium funiculosum	75	7.1			25	11.1		
Penicillium meleagrinum					25	11.1		
Penicillium notatum					25	11.1		
Penicillium velutinum			25	11.1				
Penicillium waksmani			25	11.1				
Phoma herbarum	75	11.9						
Phoma violacea	50	14.2	25	11.1				
Sporotrichum chlorinum	25	2.3						
Sterile Mycelia	50	26.1	25	11.1	50	22.2	25	100
Trichoderma viride	25	9.5						
Total Isolates	42		9		9		1	
Total Species	15		6		8		1	

TABLE 26

Site 2 - Assay for September 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	50	42.1	50	36.3				
Aspergillus fumigatus			50	27.2	50	66.6	25	66.6
Chaetomium funicola	25	10.5						
Oospora sulphurea	25	5.2						
Penicillium cyclopium	25	5.2			25	33.3		
Penicillium jenseni			25	9				
Penicillium tardum	25	5.2						
Phoma herbarum			25	18.1				
Sterile Mycelia	50	26.3	25	9			25	33.3
Trichoderma viride	25	5.2						
Total Isolates	19		11		3		3	
Total Species	7		5		2		2	

TABLE 27

Site 2 - Assay for November 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus							25	6.2
Aureobasidium pullulans	25	6.4						
Botrytis cinerea							25	6.2
Candida species	25	6.4						
Cephalosporium species	25	3.2						
Cladosporium cladosporioides					25	20		
Epicoccum purpurascens	25	3.2						
Fusarium sambucinum var. caeruleum	50	9.6						
Fusarium species					25	20		
Mucor lausanensis			25	20				
Mucor microsporus	25	3.2					25	12.5
Penicillium cyclopium					50	40		
Penicillium lanosum	25	3.2						
Penicillium ochraceum			25	20			25	6.2
Penicillium simplicissimum	25	3.2					25	6.2
Phoma glomerata	25	3.2			25	20	25	6.2
Phoma herbarum	50	9.6						
Phoma species	75	22.5						
Sterile Mycelia	100	12.9	50	40			100	56.2
Trichoderma viride	25	9.6						
Verticillium candelabrum	25	3.3						
Yeast species			25	20				
Total Isolates	31		5		5		16	
Total Species	14		4		4		7	

TABLE 28

Site 2 - Assay for January 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus			25	20				
Coniothyrium fuckelii	25	20	25	20				
Penicillium brevi-compactum	25	20	25	20				
Penicillium frequentans	25	40			25	100		
Penicillium funiculosum							25	100
Penicillium martensii			25	20				
Penicillium melinii	25	20						
Sterile Mycelia			25	20				
Total Isolates	5		5		2		1	
Total Species	4		5		1		1	

TABLE 29

Site 2 - Assay for March 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus chevalieri	50	33.3						
Aspergillus chevalieri var. intermedius	50	33.3						
Aspergillus fumigatus			50	50				
Aspergillus glaucus	50	33.3	50	50				
Penicillium ochraceum							50	100
Total Isolates	3		2				1	
Total Species	3		2				1	

TABLE 30

Site 2 - Assay for May 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	3.8						
Aspergillus fumigatus	25	3.8						
Chaetomium globosum	25	7.6						
Cladosporium cladosporioides	25	3.8	25	50				
Coniothyrium fuckelii	25	3.8						
Epicoccum purpurascens	25	3.8						
Penicillium cyclopium	50	11.5						
Penicillium expansum	25	3.8						
Penicillium funiculosum	50	7.6					25	50
Penicillium martensii	25	3.8						
Phoma glomerata	50	7.6			25	100	25	50
Phoma herbarum	75	19.2						
Sterile Mycelia	25	7.6						
Trichoderma viride	50	11.5	25	50				
Total Isolates	26		2		1		2	
Total Species	14		2		1		2	

TABLE 31

Site 2 - Assay for July 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria humicola			25	25				
Alternaria tenuis	50	3.7	25	25	50	66.6	75	40
Arthrinium phaeospermum	25	1.8						
Aspergillus fumigatus	50	3.7						
Aureobasidium pullulans	25	1.8						
Cephalosporium curtipes			25	25				
Chaetomium homopilatum	25	3.7						
Cladosporium cladosporioides							25	10
Cladosporium herbarum	25	1.8						
Coniothyrium fuckelii	50	7.5						
Epicoccum purpurascens	50	3.7						
Fusarium solani	25	1.8						
Geotrichum candidum	25	1.8						
Penicillium cyclopium	25	3.7					25	20
Penicillium frequentans	50	5.6						
Penicillium janthinellum	25	1.8						
Penicillium martensii							25	10
Penicillium notatum	25	3.7						
Penicillium ochro-chloron	25	1.8						
Penicillium palitans	50	3.7						
Penicillium spinulosum							25	10
Penicillium urticae	25	1.8						
Phoma glomerata	50	5.6						
Phoma herbarum	100	18.8						
Sterile Mycelia	75	7.5	25	25	25	33.3	25	10
Torula herbarum	25	1.8						
Trichoderma viride	50	11.3						
Total Isolates	53		4		3		10	
Total Species	22		4		2		6	

TABLE 32

Site 2 - Assay for September 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria tenuis					25	100	25	50
Aspergillus fumigatus			25	20				
Botrytis cinerea	25	4.1						
Coniothyrium fuckelii	25	4.1						
Epicoccum purpurascens			25	20				
Fusarium solani	25	8.3						
Mucor hiemalis			25	20				
Penicillium citrinum	25	4.1						
Penicillium funiculosum	25	4.1						
Penicillium simplicissimum	25	8.3						
Phoma herbarum	50	8.3	25	20				
Spicaria violacea	25	4.1						
Sterile Mycelia	50	54.1	25	20			25	50
Total Isolates	24		5		1		2	
Total Species	9		5		1		2	

TABLE 33

Site 2 - Assay for November 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia butleri	25	9	25	28.5				
Aspergillus fumigatus	50	18.1	25	14.2	25	100	25	16.6
Aspergillus glaucus	25	4.5						
Cephalosporium acremonium	25	4.5						
Chaetomium homopilatum			25	14.2				
Coniothyrium fuckelii	25	18.1	25	42.8				
Mucor hiemalis	25	4.5					25	33.3
Penicillium purpurescens	25	4.5						
Phoma humicola							25	16.6
Sporotrichum roseolum	25	4.5						
Sterile Mycelia	50	13.6						
Trichoderma viride	25	13.6						
Verticillium candelabrum	25	4.5						
Yeast species							25	33.3
Total Isolates	22		7		1		6	
Total Species	11		4		1		4	

TABLE 34

Site 2 - Assay for January 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	50	8			25	7.6		
Cephalosporium acremonium	25	4						
Cephalosporium roseo-griseum	25	4						
Coniothyrium fuckelii	50	16	25	11.1			50	50
Epicoccum purpurascens			25	11.1	25	7.6		
Mucor globosus	25	4						
Mucor hiemalis			25	22.2				
Mucor racemosus	25	4						
Mucor varians	25	4						
Penicillium claviforme	25	4						
Penicillium cyclopium	25	4						
Penicillium frequentans	25	4			25	7.6		
Penicillium funiculosum					25	7.6		
Penicillium humuli			25	11.1				
Penicillium lilacinum					25	7.6		
Penicillium restrictum			25	11.1				
Phoma herbarum	25	4			50	30.7	25	25
Sterile Mycelia	75	12	50	33.3	50	30.7	25	25
Trichoderma viride	50	20						
Verticillium candelabrum	25	8						
Total Isolates	25		9		13		8	
Total Species	14		6		7		3	

TABLE 3.5

Site 2 - Assay for March 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	50	18.7	50	25	25	33.3		
Aspergillus niger			25	12.5				
Coniothyrium fuckelii	25	6.2						
Penicillium citrinum	25	6.2			25	33.3		
Penicillium cyaneo-fulvum					25	33.3		
Penicillium cyclopium	25	6.2	25	12.5				
Penicillium frequentans			25	12.5				
Penicillium funiculosum			25	37.5				
Penicillium notatum	25	12.5						
Penicillium spinulosum	25	6.2						
Phoma herbarum	50	18.7						
Sterile Mycelia	75	25						
Total Isolates	16		8		3			
Total Species	8		5		3			

TABLE 36

Lead analysis pH and soil moisture measurements of soil samples from Site 2

Sample		Lead analysis		Top Soil		5cm		15cm		25cm	
		HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	H_2O
May	1970	14,000	16,400	7.1	20.0	7.0	24.0	6.2	23.0	6.8	20.0
July		16,000	17,960	8.4	21.0	8.5	19.0	8.3	20.0	8.3	22.0
September		15,200	16,800	7.0	22.0	7.1	27.0	6.9	24.0	7.0	25.0
November		11,600	14,200	6.8	21.0	6.6	26.0	6.6	16.0	6.6	19.0
January	1971	27,200	30,000	7.7	30.0	7.9	31.0	8.1	32.0	8.1	31.0
March		16,200	17,200	7.4	27.0	7.6	27.0	7.5	26.0	7.5	28.0
May		17,000	19,200	7.3	23.0	7.2	10.0	7.2	25.0	7.3	23.0
July		19.000	20,400	7.4	30.0	7.3	24.0	7.1	27.0	7.2	25.0
September		7,400	7,600	8.6	25.0	8.5	27.0	8.4	26.0	8.6	17.0
November		15,600	16,400	7.7	31.0	8.1	29.0	8.2	28.0	8.4	28.0
January	1972	16,200	24,000	8.1	11.5	8.1	10.0	8.1	13.5	8.1	11.0
March		16,600	28,000	8.2	27.5	8.3	27.0	8.4	31.0	8.4	27.5
May		8,000	19,600	7.4	6.5	7.4	20.0	7.4	19.0	7.5	15.0

TABLE 37

Site 3 - Assay for May 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	50	12.9			50	57.1	50	50
Coniothyrium fuckelii	50	6.4						
Emericellopsis species	50	6.4						
Mucor hiemalis	50	25.8						
Oospora species	25	3.2						
Penicillium funiculosum			25	33.3			50	50
Penicillium notatum					25	14.2		
Phoma herbarum	75	25.8						
Phomopsis species	50	16.1	25	33.3				
Sordaria fimicola					25	14.2		
Sterile Mycelia	25	3.2			25	14.2		
Verticillium bulbillosum			25	33.3				
Total Isolates	31		3		7		4	
Total Species	8		3		4		2	

TABLE 38

Site 3 - Assay for July 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea							50	25
Aspergillus fumigatus	50	10						
Aspergillus repens	25	5						
Botrytis cinerea					25	33.3	25	8.3
Candida species	25	5						
Cylindrocarpon heteronemum							25	8.3
Emericellopsis species	25	5						
Epicoccum purpurascens	25	5			25	33.3		
Oospora sulphurea	25	10						
Penicillium brevi-compactum			25	42.8				
Penicillium implicatum							25	8.3
Penicillium jenseni			25	14.2				
Penicillium ochro-chloron	25	10	25	14.2				
Penicillium variabile	25	5						
Phoma violacea	25	5					25	25
Preussia vulgare	50	10					25	16.6
Scopulariopsis brevicaulis					25	33.3		
Sterile Mycelia	50	30					25	8.3
Verticillium psalliotae			25	14.2				
Verticillium terrestre			25	14.2				
Total Isolates	20		7		3		12	
Total Species	11		5		3		7	

TABLE 39

Site 3 - Assay for September 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	8.6					25	19
Arthrinium phaeospermum			25	25				
Aspergillus amstelodami							25	9.5
Aspergillus fumigatus	25	4.3	25	50	50	50	50	9.5
Candida species	25	4.3					25	4.7
Coniothyrium fuckelii							25	4.7
Fusarium species					25	12.5		
Mucor corticolus	25	4.3						
Penicillium cyclopium							25	4.7
Penicillium notatum							25	4.7
Penicillium paxilli	25	4.3			50	25		
Phoma herbarum	100	47.8	25	25			100	23.8
Preussia vulgare	50	8.6					25	4.7
Sterile mycelia	50	13			25	12.5	75	14.2
Verticillium candelabrum	25	4.3						
Total Isolates	23		4		8		21	
Total Species	9		3		4		10	

TABLE 40

Site 3 - Assay for November 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus			25	22.2	50	50	25	25
Aspergillus species	25	2.7	25	11.1				
Aureobasidium pullulans	25	2.7						
Candida species	25	2.7						
Coniothyrium fuckelii	50	19.4	25	11.1				
Fusarium culmorum	25	2.7						
Penicillium brevi-compactum			25	11.1				
Penicillium corymbiferum					25	12.5		
Penicillium cyclopium			50	22.2	50	37.5		
Penicillium chrysogenum							25	12.5
Penicillium funiculosum							25	12.5
Penicillium ochraceum	25	2.7	25	11.1				
Penicillium simplicissimum							25	12.5
Phoma glomerata	25	5.5	25	11.1				
Phoma herbarum	100	44.4					50	37.5
Sterile Mycelia	75	16.6						
Total Isolates	36		9		8		8	
Total Species	9		7		3		5	

TABLE 41

Site 3 - Assay for January 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Arthrinium phaeospermum	25	4.1						
Aspergillus fumigatus	75	12.5	25	7.1	25	33.3	25	60
Coniothyrium fuckelii	50	12.5	25	7.1				
Cylindrocarpon candidum	25	4.1						
Fusarium species	25	4.1						
Mucor spinescens			25	14.2				
Paecilomyces varioti			25	14.2				
Penicillium cyclopium	25	4.1						
Penicillium frequentans	25	12.5	75	21.4				
Penicillium funiculosum			25	7.1				
Penicillium lanosum			25	14.2				
Penicillium melinii					25	33.3		
Penicillium meleagrinum	25	4.1						
Penicillium notatum							25	20
Penicillium spinulosum			25	7.1			25	20
Phoma herbarum	50	20.8	25	7.1				
Scopulariopsis brevicaulis					25	33.3		
Sterile Mycelia	50	16.6						
Yeast species	25	4.1						
Total Isolates	24		14		3		5	
Total Species	11		9		3		3	

TABLE 42

Site 3 - Assay for March 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus chevalieri var. intermedius	25	50			25	11.1		
Aspergillus fumigatus	25	50	25	50	25	11.1	25	28.5
Aspergillus glaucus					25	55.5		
Penicillium frequentans							25	28.5
Penicillium funiculosum			25	50	25	11.1	25	14.2
Penicillium notatum							25	28.5
Sterile Mycelia					25	11.1		
Total Isolates	2		6		9		7	
Total Species	2		2		5		4	

TABLE 43

Site 3 - Assay for May 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	50	6	25	11.1				
Aureobasidium pullulans			25	3.7				
Cladosporium cladosporioides					25	60		
Coniothyrium fuckelii							25	20
Fusarium culmorum	25	3						
Fusarium sambucinum var. caeruleum	75	24.2						
Fusarium species	25	6						
Penicillium citrinum					25	20		
Penicillium ochro-chloron							25	20
Penicillium simplicissimum							25	40
Penicillium viridicatum			50	11.1			25	20
Phoma glomerata			25	3.7				
Phoma herbarum	100	42.4	75	18.5				
Sphaeronaema spinella	25	3						
Sterile Mycelia	50	9	50	48.1	25	20		
Yeast species	25	6	25	3.7				
Total Isolates	33		27		5		5	
Total Species	8		7		3		4	

TABLE 44

Site 3 - Assay for July 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Alternaria humicola	25	2						
Aspergillus fumigatus	50	4						
Cephalosporium curtipes							25	10
Cladosporium cladosporioides			25	16.6	25	7.1	25	10
Cladosporium herbarum	25	2			50	21.4	25	10
Cylindrocarpon olidum			25	16.6				
Epicoccum purpurascens	25	4						
Fusarium solani	25	2						
Penicillium fellutanum	25	2						
Penicillium jenseni			25	16.6				
Penicillium spinulosum	75	6					25	10
Phoma glomerata	75	22					50	20
Phoma herbarum	100	50					50	20
Sporobolomyces roseus					25	7.1		
Sporotrichum olivaceum			25	16.6	75	57.1	50	20
Sterile Mycelia	25	2	25	33.3	25	7.1		
Verticillium candelabrum	25	2						
Yeast species	25	2						
Total Isolates	50		6		14		10	
Total Species	12		5		5		7	

TABLE 45

Site 3 - Assay for September 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	25	11.7	25	9	25	14.2	25	14.2
Aureobasidium pullulans	25	11.7						
Cephalosporium curtipes			25	9				
Coniothyrium fuckelii	50	23.5						
Cylindrocarpon olidum			25	36.3	25	14.2		
Epicoccum purpurascens			25	9				
Penicillium cyclopium			25	9				
Penicillium spinulosum	25	5.8						
Phoma herbarum					25	14.2	25	14.2
Scopulariopsis constantini					25	28.5		
Sterile Mycelia	100	41.1	75	27.2	50	28.5	75	71.4
Yeast species	25	5.8						
Total Isolates	17		11		7		7	
Total Species	6		6		5		3	

TABLE 46

Site 3 - Assay for November 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia butleri	50	31.2					25	19
Aspergillus fumigatus	100	37.5					25	4.7
Aureobasidium pullulans							25	9.5
Cephalosporium acremonium			25	7.1				
Cephalosporium humicola			25	21.4				
Coniothyrium fuckelii	50	12.5					75	14.2
Graphium penicillioides							25	4.7
Penicillium cyclopium							25	4.7
Penicillium purpurogenum			25	7.1				
Phoma herbarum							25	4.7
Sordaria fimicola	25	6.2					50	19
Sterile Mycelia	25	6.2	50	50	25	14.2	75	19
Yeast species	25	6.2	50	14.2	50	85.7		
Total Isolates	16		14		7		21	
Total Species	6		5		2		9	

TABLE 47

Site 3 - Assay for January 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus					25	4.3	100	25
Aspergillus glaucus					25	4.3		
Aureobasidium pullulans	25	6						
Candida albicans					25	4.3		
Coniothyrium fuckelii	50	9			25	4.3	75	18.7
Epicoccum purpurascens	25	3					25	18.7
Mucor globosus					25	4.3		
Mucor hiemalis	25	15.1						
Penicillium claviforme					25	4.3		
Penicillium cyclopium					25	8.6		
Penicillium frequentans					50	13	50	12.5
Penicillium funiculosum					25	4.3		
Penicillium janthinellum	25	3						
Penicillium thomii			25	33.3	25	13		
Phoma glomerata					25	4.3		
Phoma herbarum	75	51.5			75	30.4	25	12.5
Sterile Mycelia			50	33.3			50	12.5
Trichoderma viride	50	9						
Verticillium sulphurellum			25	16.6				
Yeast species	25	3	25	16.6				
Total Isolates	33		6		23		16	
Total Species	8		4		12		6	

TABLE 48.

Site 3 - Assay for March 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	50	12.7	75	95.4	25	61.1	100	85.7
Aspergillus niger	25	2.1						
Cephalosporium acremonium	25	2.1						
Coniothyrium fuckelii	25	2.1	25	4.5				
Fusarium sambucinum var. caeruleum	50	12.7						
Fusarium solani	25	4.2						
Mucor hiemalis	50	4.2						
Penicillium brevi-compactum					25	5.5		
Penicillium canescens					25	5.5		
Penicillium cyclopium					25	5.5	25	7.1
Penicillium frequentans	25	2.1						
Penicillium funiculosum	25	2.1						
Penicillium lilacinum	25	6.3						
Penicillium viridicatum					25	5.5	25	7.1
Phoma herbarum	100	27.6			50	11.1		
Scopulariopsis brevicaulis					25	5.5		
Sterile Mycelia	50	21.2						
Total Isolates	47		22		18		14	
Total Species	12		2		7		3	

TABLE 49

Lead analysis pH and soil moisture measurements of soil samples from Site 3

Sample	Lead analysis		Top Soil		5cm		15cm		25cm	
	HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
May 1970	16,400	28,400	6.6	19.0	6.4	12.0	7.0	11.0	6.3	6.3
July	28,400	45,600	8.6	2.6	8.5	11.0	8.5	14.0	8.5	11.0
September	27,200	44,000	6.8	12.0	6.7	12.0	6.8	16.0	6.5	12.0
November	12,400	29.600	6.8	17.0	6.8	18.0	6.8	17.0	6.8	15.0
January 1971	32,000	58,400	7.4	15.0	7.5	16.0	7.3	19.0	7.3	17.0
March	11,200	35,600	7.6	17.0	7.4	20.0	7.2	24.0	7.3	14.0
May	22,000	56,000	7.3	10.0	7.4	9.5	7.3	12.0	7.3	11.0
July	17,000	24,400	7.2	14.0	7.3	17.0	7.4	12.0	7.3	15.0
September	24,400	39.200	7.6	17.0	7.4	14.0	7.5	12.0	7.5	26.0
November	26,400	37,600	7.1	22.0	7.2	18.0	7.1	18.0	7.4	20.0
January 1972	11,600	22,800	7.4	6.0	7.6	3.5	7.6	4.0	7.8	3.5
March	29,600	29,600	7.7	16.5	7.8	17.5	7.9	22.5	7.8	19.5
May	32,800	32,000	7.3	12.5	7.4	10.0	7.4	11.0	7.4	10.0

TABLE 50

Site 4 - Assay for May 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus	75	19.2	25	11.1	25	28.5		
Aspergillus niger	25	3.8						
Cephalosporium acremonium			25	11.1				
Cephalosporium roseo-griseum			25	11.1				
Chaetomium funicola	25	3.8						
Coniothyrium fuckelii	50	7.6	25	11.1	25	14.2		
Cylindrocarpon olidum	50	7.6						
Emericellopsis species					50	28.5		
Gliocladium penicilloides	25	3.8						
Humicola brevis			25	11.1				
Penicillium citreo-viride			25	11.1				
Penicillium jenseni			25	11.1				
Penicillium lilacinum	75	19.2						
Penicillium roseo-purpureum			25	11.1				
Phoma herbarum	50	19.2			50	28.5		
Sterile Mycelia	50	7.6	25	11.1				
Trichoderma viride	25	7.6						
Total Isolates	26		9		7			
Total Species	10		9		4			

TABLE 5 1.

Site 4 - Assay for July 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea			50	11.1	25	2.3		
Aspergillus fumigatus	25	2.8			25	2.3		
Botrytis cinerea					25	2.3		
Mammaria echinobotryoides			25	3.7				
Mucor griseo-lilacinus	25	2.8						
Mucor varians	25	2.8	25	7.4	25	4.7		
Penicillium brevi-compactum			25	3.7				
Penicillium funiculosum			25	3.7				
Penicillium lilacinum			25	3.7	100	45.2		
Penicillium oxalicum					25	2.3		
Penicillium tardum					25	2.3		
Phoma herbarum	50	8.5	75	18.5	50	7.1		
Phoma violacea	50	25.7	50	40.7	50	23.8		
Rhizopus oryzae	25	2.8						
Sterile Mycelia	100	54.2	50	7.4	50	23.8		
Total Isolates	35		27		42			
Total Species	7		9		10			

TABLE 52

Site 4 - Assay for September 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	50	8.8	25	6.2				
Absidia spinosa	25	5.8						
Aspergillus fumigatus			25	6.2	25	12.1		
Botryotrichum atrogriseum	50	20.5						
Epicoccum purpurascens					25	3		
Gliocladium roseum			25	12.5				
Oospora sulphurea			25	6.2	25	3		
Penicillium chrysogenum			25	6.2				
Penicillium cyclopium			25	6.2				
Penicillium godlewskii					25	6		
Penicillium lilacinum	100	35.2			25	12.1		
Penicillium ochro-chloron			25	6.2				
Phoma glomerata			25	6.2				
Phoma herbarum	75	20.5	75	43.7	100	45.4		
Sterile Mycelia	25	8.8			75	18.1		
Total Isolates	34		16		33			
Total Species	6		9		7			

TABLE 53

Site 4 - Assay for November 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus fumigatus					25	7.1		
Candida species	25	2.7						
Coniothyrium fuckelii			25	5.2				
Cylindrocarpon olidum	25	8.3						
Fusarium culmorum	25	2.7						
Fusarium oxysporum			25	5.2				
Fusarium sambucinum var. caeruleum	25	2.7	50	15.7				
Fusarium solani	25	2.7	25	5.2				
Gliocladium roseum	25	2.7			50	28.5		
Gliocladium salmonicolor	25	5.5			25	7.1		
Mucor corticolus			25	5.2				
Mucor hiemalis	25	5.5						
Mucor microsporus	50	16.6	50	15.7				
Penicillium canescens			25	5.2				
Penicillium chrysogenum			25	5.2				
Penicillium funiculosum					50	21.4		
Penicillium humuli	25	2.7	50	10.5	25	14.2		
Penicillium lilacinum	25	2.7	25	5.2				
Penicillium meleagrinum	25	2.7						
Penicillium notatum					25	7.1		
Penicillium piscarium	25	2.7						
Penicillium simplicissimum	25	2.7						
Phoma herbarum			50	10.5				
Sterile Mycelium	50	11.1			25	7.1		
Trichoderma viride	75	16.6	25	5.2	25	7.1		
Verticillium effusum	25	2.7	25	5.2				
Zygorhynchus vuilleminii	50	5.5						
Total Isolates	36		19		14			
Total Species	18		13		8			

TABLE 54

Site 4 - Assay for January 1971

SPECIES	ADDED - TOP SOIL		TOPSOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia spinosa	25	1.6						
Chaetomium homopilatum	25	1.6						
Chaetomium globosum	25	1.6						
Coniothyrium fuckelii	50	6.7						
Fusarium culmorum	50	3.3						
Fusarium oxysporum					25	6.8		
Fusarium sambucinum var. caeruleum	25	3.3			25	3.4		
Gliocladium roseum	75	6.7	50	10	25	3.4		
Mucor abundans			25	2.5				
Mucor microsporus	50	11.8	25	2.5				
Mucor racemosus	25	3.3						
Mucor spinescens	50	8.4	50	15	25	13.7		
Mucor varians	25	1.6						
Paecilomyces varioti			50	5	25	3.4		
Penicillium canescens	25	1.6	25	2.5				
Penicillium decumbens			50	5				
Penicillium humuli	50	11.8	50	10	25	3.4		
Penicillium jenseni					25	3.4		
Penicillium melinii	50	3.3						
Penicillium ochraceum					25	3.4		
Penicillium ochro-chloron	50	3.3	50	5	25	3.4		
Penicillium piscarium			50	5	25	3.4		
Penicillium restrictum			25	2.5				
Penicillium simplicissimum	50	5	50	7.5	25	3.4		
Penicillium velutinum	25	3.3						
Penicillium species					25	6.8		
Phoma herbarum			25	2.5				

TABLE 54

Site 4

SPECIES	ADDED TOP SOIL		TOP SOIL 5cm		5 cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Rhizopus arrhizus					25	3.4		
Sepedonium chrysospermum	25	1.6						
Sterile Mycelia	50	3.3	25	2.5	50	6.8		
Trichoderma viride	75	15.2	100	17.5	100	31		
Verticillium sulphurellum			25	2.5				
Zygorhynchus moelleri			25	2.5				
Total Isolates	59		40		29			
Total Species	20		17		15			

TABLE 55

Site 4 - Assay for March 1971

SPECIES	ADDED TOP SOIL		TOP SOIL - 5cm		5 cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Ascomycetous species					25	18.1		
Aspergillus chevalieri var. intermedius			25	14.2				
Aspergillus glaucus	25	3.7						
Eladia saccula	75	12.9						
Fusarium culmorum	25	1.8						
Penicillium funiculosum	75	5.5			25	18.1		
Penicillium luteum	100	31.4	50	14.2	50	27.2		
Penicillium velutinum	25	3.7						
Sterile Mycelia	100	36.9	100	71.4	50	36.3		
Trichoderma viride	25	3.7						
Total Isolates	54		14		11			
Total Species	8		3		4			

TABLE 56

Site 4 - Assay for May 1971

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5 cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	1.2			25	6.2		
Absidia glauca					25	6.2		
Absidia spinosa			50	7.8	25	12.5		
Botrytis cinerea			25	2.6				
Coniothyrium fuckelii	25	1.2						
Doratomyces stemonitis					50	12.5		
Epicoccum purpurascens	25	1.2						
Fusarium culmorum	25	1.2						
Fusarium oxysporum	25	2.4						
Fusarium sambucinum var. caeruleum	75	6.1						
Gliocladium roseum			25	5.2				
Mucor microsporus	50	6.1						
Mucor spinescens	25	4.9			25	6.2		
Mucor varians	50	2.4						
Oospora sulphurea	25	1.2	25	2.6				
Penicillium citrinum			25	2.6				
Penicillium funiculosum			25	2.6				
Penicillium jenseni	50	2.4	25	2.6				
Penicillium melinii	25	1.2						
Penicillium nigricans			25	2.6				
Penicillium notatum					25	6.2		
Penicillium ochro-chloron	100	8.6	75	31.5				
Penicillium palitans			25	2.6				
Penicillium restrictum	25	1.2			25	6.2		
Penicillium simplicissimum			25	2.6	25	12.5		
Penicillium viridicatum					25	6.2		
Penicillium waksmani	25	2.4						

TABLE 56

Site 4

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Phoma herbarum			25	2.6	25	12.5		
Sterile Mycelia	50	2.4	50	5.2	50	12.5		
Trichoderma viride	100	46.9	75	26.3				
Yeast species	25	1.2						
Zygorhynchus vuilleminii	25	2.4						
Total Isolates	81		38		16			
Total Species	19		14		11			

TABLE 57

Site 4 - Assay for July 1971

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia heterospora	25	1.2						
Absidia spinosa	25	1.2	25	1.2				
Alternaria tenuis					25	4.7		
Cladosporium herbarum					25	4.7		
Coniothyrium fuckelii					25	4.7		
Eupenicillium species			75	3.8				
Fusarium culmorum	25	1.2						
Fusarium oxysporum	25	1.2	25	1.2				
Fusarium sambucinum var. caeruleum			25	1.2	50	19		
Fusarium solani					25	9.5		
Gliocladium roseum					25	4.7		
Mucor corticolus			25	1.2				
Mucor hiemalis	50	2.5	50	7.7	25	9.5		
Mucor varians			50	7.7				
Penicillium cyaneo-fulvum			25	1.2				
Penicillium janthinellum	75	10	50	3.8	25	4.7		
Penicillium nigricans			25	1.2				
Penicillium notatum	25	1.2	25	2.5				
Penicillium ochro-chloron	50	2.5	25	2.5	50	14.2		
Penicillium raistrickii	25	1.2	25	1.2				
Penicillium simplicissimum	100	17.5	75	5.1				
Penicillium spinulosum			25	1.2				
Penicillium terrestre	75	7.5	25	1.2	25	4.7		
Penicillium velutinum	25	1.2	25	1.2	25	4.7		
Penicillium waksmani	50	6.2	50	7.7	50	14.2		
Penicillium species	50	8.7	75	9				
Phoma herbarum			25	1.2				

TABLE 57

Site 4

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Rhizopus arrhizus	50	8.7						
Sterile Mycelia	25	1.2	50	3.8				
Trichoderma viride	75	26.2	100	24.6				
Zygorhynchus moelleri			25	6.4				
Total Isolates	80		77		21			
Total Species	17		23		12			

TABLE 58

Site 4 - Assay for September 1971

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia glauca			25	4.5				
Alternaria humicola					25	4		
Alternaria tenuis	50	7.1	75	13.6	75	6		
Aspergillus fumigatus	25	2.3	25	4.5				
Aspergillus niger					25	2		
Aureobasidium pullulans			25	4.5	50	4		
Candida albicans	25	2.3						
Cephalosporium acremonium			25	4.5				
Coniothyrium fuckelii	50	9.5	50	18.1	75	12		
Cylindrocarpon olidum					25	2		
Epicoccum purpurascens					75	8		
Fusarium sambucinum var. caeruleum			25	4.5	50	4		
Gliocladium roseum	75	21.4	50	18.1	50	6		
Monodictys levis			25	9				
Mucor hiemalis	25	4.7						
Paecilomyces carneus	25	2.3						
Paecilomyces striatosporus					25	2		
Penicillium canescens					25	4		
Penicillium janthinellum	25	2.3						
Penicillium jenseni					25	2		
Penicillium lilacinum	25	2.3			25	6		
Penicillium notatum					25	4		
Penicillium purpurogenum					25	2		
Penicillium thomii					25	2		
Phoma herbarum					25	2		
Sterile Mycelia	25	4.7	50	13.6	100	8		
Trichoderma viride	100	38	25	4.5	75	16		

TABLE 58

Site 4

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Verticillium terrestre	25	2.3						
Yeast species					25	2		
Total Isolates	42		22		50			
Total Species	12		11		20			

TABLE 59

Site 4 - Assay for November 1971

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia spinosa	25	4.7						
Alternaria tenuis	25	9.5						
Aspergillus fumigatus			25	6.3	25	3.8		
Aspergillus glaucus					25	3.8		
Aureobasidium pullulans			25	4.2				
Cephalosporium acremonium	25	4.7	25	2.1				
Cephalosporium curtipes			50	8.5				
Cephalosporium humicola					25	3.8		
Coniothyrium fuckelii			100	36.1	75	19.2		
Fusarium sambucinum var. caeruleum	25	4.7						
Geotrichum candidum					25	3.8		
Gliocladium roseum			50	14.8				
Graphium penicillioides			25	2.1	50	7.6		
Mucor hiemalis	50	23.8	25	2.1				
Oospora sulphurea			25	2.1				
Penicillium brevi-compactum					25	3.8		
Penicillium citrinum					25	3.8		
Penicillium frequentans					25	11.5		
Penicillium humuli			25	2.1				
Penicillium javanicum	25	9.5						
Penicillium lilacinum			25	4.2				
Penicillium nigricans	25	4.7						
Penicillium purpurrescens			25	2.1				
Penicillium purpurogenum			25	2.1				
Penicillium restrictum			25	2.1				
Penicillium simplicissimum	25	4.7						

TABLE 59

Site 4

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Pestalotia pezizoides	25	4.7						
Phoma herbarum			50	4.2	25	11.5		
Sordaria fimicola					25	7.6		
Sterile Mycelia	25	9.5			75	19.2		
Trichoderma viride	50	14.2	25	4.2				
Zygorhynchus heterogamus	25	4.7						
Total Isolates	21		47		26			
Total Species	12		16		12			

TABLE 60

Site 4 - Assay for January 1972

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia spinosa			25	2.4				
Aspergillus fumigatus	25	5.1	25	2.4	25	13.6		
Aspergillus ochraceus					25	4.5		
Aureobasidium pullulans					25	4.5		
Coniothyrium fuckelii	25	3.4	25	4.8				
Cylindrocarpon candidum					25	4.5		
Cylindrocarpon olidum			25	2.4	25	4.5		
Epicoccum purpurascens			25	2.4				
Fusarium sambucinum var. caeruleum	25	10.3	50	7.3	25	4.5		
Gliocladium roseum	100	22.4	50	4.8	25	9		
Mucor hiemalis	50	5.1	25	4.8				
Penicillium decumbens	25	1.7						
Penicillium frequentans			25	2.4	25	9		
Penicillium funiculosum					50	18.1		
Penicillium janthinellum	25	1.7	25	2.4				
Penicillium luteum					25	4.5		
Penicillium thomii			25	2.4				
Phoma herbarum	25	5.1	50	12.1	25	4.5		
Sterile Mycelia	75	6.8			75	18.1		
Trichoderma viride	100	37.9	100	48.7				
Total Isolates	58		41		22			
Total Species	10		13		12			

TABLE 61

Site 4 - Assay for March 1972

SPECIES	ADDED - TOP SOIL		TOP SOIL - 5cm		5cm - 15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	1.4						
Absidia spinosa	25	1.4	25	4				
Aspergillus fumigatus	25	4.2	25	20	50	15.3		
Eladia saccula					25	3.8		
Fusarium sambucinum var. caeruleum	25	1.4	25	4	25	3.8		
Gliocladium roseum	25	1.4	50	32	50	15.3		
Monodictys levis					25	3.8		
Mucor hiemalis	75	5.7						
Mucor jansseni	25	1.4						
Paecilomyces varioti					25	3.8		
Penicillium canescens	25	1.4						
Penicillium citrinum			25	4				
Penicillium cyaneo-fulvum			25	4				
Penicillium cyclopium					25	3.8		
Penicillium daleae	25	1.4						
Penicillium frequentans	25	1.4						
Penicillium funiculosum	50	2.8	25	4	50	7.6		
Penicillium lilacinum					50	7.6		
Pencillium luteum					25	3.8		
Penicillium nigricans	75	4.2						
Penicillium restrictum	25	1.4						
Penicillium rugulosum	25	1.4						
Penicillium velutinum	25	2.8						
Sterile Mycelia	75	14.2	25	4	25	3.8		
Trichoderma viride	100	50	50	24	75	23		
Yeast species					25	3.8		
Zygorhynchus vuilleminii	25	1.4						
Total Isolates	70		25		26			
Total Species	18		9		13			

TABLE 62

Lead analysis pH and soil moisture measurements of soil samples from Site 4

Sample		Lead analysis		Top Soil Added		Top Soil		5cm		15cm	
		HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
May	1970	6,500	14,800			6.3	5.2	7.5	5.7	7.1	11.7
July		8,500	44,000			8.4	6.5	8.5	10.4	8.5	6.6
September		16,000	27,600			7.1	19.0	7.5	6.5	7.4	6.2
November		900	4,800			6.4	36.0	6.6	10.0	6.8	17.0
January	1971	1,200	19,200	5.2	31.0	7.1	14.0	7.9	4.5		
March		1,900	14,400	7.1	23.0	7.6	7.5	7.5	5.2		
May		2,450	6,000	6.6	23.0	7.2	6.3	7.2	5.1		
July		2,500	5,100	7.3	21.0	7.4	10.0	7.5	5.6		
September		2,750	16,400	8.1	6.5	7.9	6.2	7.4	6.0		
November		2,450	15,800	7.2	21.0	7.9	8.0	8.0	9.4	8.1	6.2
January	1972	2,300	7,100	6.8	4.0	7.2	6.4	7.0	2.5	7.0	2.0
March		2,000	5,000	7.4	15.5	7.6	8.0	7.5	6.0	7.7	8.5
May		700	9,100	7.5	13.0	7.5	2.5	7.6	2.0	7.6	0.5

TABLE 63

Site 5 - Assay for May 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia glauca			25	3.2	50	14.2		
Absidia heterospora			25	3.2				
Absidia spinosa			25	1.6				
Alternaria fasciculata			25	1.6				
Alternaria humicola							25	2
Aspergillus fumigatus					25	2.3	25	2
Aspergillus niger			25	1.6				
Cephalosporium curtipes	50	3.3	25	4.9			25	2
Cephalosporium roseo-griseum	25	1.6						
Fusarium culmorum	75	11.8	50	11.4	25	2.3	50	8.1
Fusarium oxysporum	25	3.3			25	2.6	25	2
Fusarium sambucinum var. caeruleum	50	3.3	75	11.4	75	16.6	50	8.1
Fusarium solani	25	1.6	25	1.6	75	9.5	25	2
Fusarium species	75	8.3	75	11.3	50	14.2	50	10.2
Gliocladium roseum	75	10.1	50	9.8	25	4.7	75	10.2
Gliocladium salmonicolor	25	1.6	25	1.6				
Monilia geophila							25	2
Mucor abundans					25	2.6		
Mucor hiemalis	25	5	25	6.5			50	6.1
Mucor jansseni			25	1.6				
Mucor microsporus	50	16.9	25	1.6				
Mucor racemosus			25	3.2			50	14.2
Mucor spinescens			25	3.2			25	2
Mucor subtillissimus	50	3.3						
Mucor varians			25	3.2				
Oospora sulphurea	25	1.6			50	9.5		
Paecilomyces varioti					25	2.3	25	2

TABLE 63

Site 5

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Penicillium canescens					25	4.7		
Penicillium cyaneo-fulvum			25	1.6				
Penicillium cyclopium			25	3.2			25	2
Penicillium frequentans	25	3.3						
Penicillium funiculosum					25	2.3		
Penicillium ochro-chloron							25	2
Penicillium simplicissimum	25	1.6					50	4
Sterile Mycelia	50	3.3	25	3.2	25	2.3	75	10.2
Trichoderma viride	75	15.2	50	3.2	50	4.7	50	4
Verticillium candelabrum			50	3.2	25	2.3		
Verticillium cinnabarinum							25	2
Zygorhynchus heterogamus			25	1.6	25	2.3		
Zygorhynchus moelleri							25	2
Zygorhynchus vuillemini	25	3.3						
Total Isolates	59		61		42		49	
Total Species	18		24		17		21	

TABLE 6.4

Site 5 - Assay for July 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea							50	3.9
Absidia glauca	25	7.5	50	8.4	25	2.5		
Absidia heterospora			25	3.3				
Absidia spinosa					25	2.5		
Aspergillus fumigatus			25	1.6	25	5.1	25	1.9
Coniothyrium fuckelii	50	5			25	2.5	25	1.9
Fusarium culmorum	50	5	100	6.7	50	5.1	50	3.9
Fusarium species	50	7.5	50	5			50	5.8
Gliocladium roseum	25	5	75	10.1	75	10.2	100	17.6
Gliocladium salmonicolor			50	3.3				
Mucor varians	25	2.5	25	5	25	5.1		
Oospora variabilis					25	2.5		
Paecilomyces varioti					25	2.5	25	1.9
Penicillium aculeatum					25	2.5		
Penicillium canescens					25	2.5	25	3.9
Penicillium claviforme							25	1.9
Penicillium crustosum					25	2.5		
Penicillium commune					25	2.5		
Penicillium decumbens	75	7.5	75	5	25	5.1	50	9.8
Penicillium frequentans			25	1.6				
Penicillium funiculosum	25	2.5					25	1.9
Penicillium jenseni	25	2.5						
Penicillium lanosum	25	2.5			25	2.5		
Penicillium lilacinum	50	5			25	2.5	25	3.9
Penicillium megasporium	25	2.5	25	1.6				
Penicillium meleagrinum	25	2.5						
Penicillium nigricans	25	2.5						
Penicillium spinulosum			25	1.6	25	7.6		

TABLE 64

Site 5

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Penicillium terrestre							25	1.9
Penicillium velutinum	25	2.5	25	1.6	25	2.5	25	1.9
Pestalotia pezizoides	25	2.5						
Pyrenochaeta decipiens					25	2.5		
Scopulariopsis brevicaulis					25	5.1		
Sterile Mycelia	25	7.5			25	2.5	25	5.8
Trichoderma viride	50	27.5	75	42.3	50	17.9	50	31.3
Verticillium candelabrum			25	1.6	25	2.5		
Total Isolates	40		59		39		51	
Total Species	18		15		23		16	

TABLE 65.

Site 5 - Assay for September 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia orchidis					25	5.1		
Aspergillus fumigatus	100	15.1	100	51.3	75	48.7	100	35.7
Aspergillus sulphureus					25	2.5		
Aspergillus terreus							25	1.7
Aspergillus versicolor	50	6	50	8.1	25	2.5	50	3.5
Botrytis cinerea			25	2.7				
Coniothyrium fuckelii	25	3						
Cylindrocarpon olidum							25	3.5
Fusarium culmorum	25	3					75	7.1
Fusarium oxysporum	25	3						
Fusarium sambucinum var. caeruleum	50	12.1	25	2.7			25	1.7
Fusarium solani							25	1.7
Gliocladium roseum			50	5.4	75	10.2	100	17.8
Gliocladium salmonicolor					25	2.5	25	1.7
Mucor varians	50	6	50	5.4	25	2.5		
Paecilomyces varioti							25	1.7
Penicillium corymbiferum	25	3						
Penicillium cyclopium	25	3						
Penicillium duclauxi	25	3					25	1.7
Rhizopus oryzae							25	3.5
Sterile Mycelia	25	3	50	5.4	25	2.5	50	3.5
Trichoderma viride	100	39.3	75	16.2	50	15.3	75	12.5
Trichoderma album			25	2.7				
Verticillium candelabrum							25	1.7
Verticillium terrestre					25	7.6		
Total Isolates	33		37		39		56	
Total Species	12		9		10		15	

TABLE 66

Site 5 - Assay for November 1970

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Arthrinium phaeospermum	25	2.5						
Aspergillus niger			25	2.7				
Aspergillus species					25	2.9		
Aureobasidium pullulans			25	2.7	25	2.9		
Candida species	50	10			25	5.8	25	7.6
Cephalosporium acremonium	25	2.5	25	2.7	50	11.7	25	7.6
Cylindrocarpon candidum			50	8.3			25	7.6
Fusarium culmorum	25	2.5	25	5.5	25	2.9	25	15.3
Fusarium sambucinum var. caeruleum	50	12.5			50	23.5		
Fusarium species	25	2.5	25	5.5	25	2.9	50	15.3
Gliocladium roseum	75	7.5	50	8.3	25	2.9		
Mucor microsporus	75	12.5	50	19.4	25	11.7	25	7.6
Oospora sulphurea					25	2.9		
Penicillium brevi-compactum	25	2.5					25	7.6
Penicillium cyclopium			25	2.7				
Penicillium ochraceum			25	2.7				
Penicillium puberulum	25	2.5					25	7.6
Penicillium rubrum	25	2.5						
Penicillium simplicissimum							25	7.6
Phoma herbarum			50	5.5				
Rhizopus oryzae	25	2.5						
Sterile Mycelia	25	2.5	50	13.8	100	11.7	25	6.6
Trichoderma viride	75	22.5	50	8.3	50	14.7		
Verticillium candelabrum	25	2.5						
Yeast species	50	10	25	11.1	25	2.9	25	7.6
Total Isolates	40		36		34		13	
Total Species	16		14		13		11	

TABLE 67

Site 5 - Assay for January 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia glauca							25	2.1
Absidia spinosa	50	10.3						
Aspergillus fumigatus	25	6.8	50	9.3	75	19.5	75	26
Aspergillus glaucus	25	3.4	25	3.1				
Candida albicans							25	2.1
Cephalosporium acremonium					25	2.4	50	4.3
Chaetomium homopilatum	25	6.8	50	28.1	50	9.7	50	8.6
Chaetomium olivaceum	25	3.4	50	6.2	25	4.8	50	8.6
Coniothyrium fuckelii					25	4.8		
Fusarium culmorum	25	3.4						
Fusarium species			25	3.1				
Gliocladium roseum			25	6.2	25	4.8		
Mucor microsporus	25	13.7			25	4.8	25	2.1
Penicillium cylopium	25	10.3	25	9.3	25	4.8	50	6.5
Penicillium decumbens	25	3.4	50	9.3	25	4.8	25	2.1
Penicillium frequentans	25	13.7						
Penicillium funiculosum			25	9.3			25	4.3
Penicillium luteum					25	2.4	25	4.3
Penicillium notatum	25	3.4						
Penicillium ochraceum	50	6.8						
Penicillium ramigena							25	2.1
Sepedonium chrysospermum							50	4.3
Sterile Mycelia	50	13.7	50	15.6	50	26.8	50	10.8
Trichoderma viride					25	9.7	75	10.8
Total Isolates	29		32		41		46	
Total Species	13		10		12		15	

TABLE 6 8

Site 5 - Assay for March 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Aspergillus chevalieri var. intermedius	50	27	75	12.9	50	21.4	50	20
Aspergillus fumigatus	25	13.5	25	3.2	75	10.7	50	20
Aspergillus glaucus	75	13.5	75	25.8	25	14.2	50	20
Aspergillus sydowi	25	2.7						
Cephalosporium acremonium	25	5.4	25	3.2	25	10.7		
Cephalosporium species	50	5.4	25	3.2	25	3.5		
Chaetomium olivaceum					25	3.5		
Coniothyrium fuckelii							25	10
Penicillium canescens	25	27	25	3.2	25	3.5		
Penicillium cyclopium			25	3.2			25	20
Penicillium decumbens			25	3.2				
Penicillium diversum					50	7.1		
Penicillium duclauxi	25	2.7	50	12.9	25	3.5		
Penicillium fellutanum	25	5.4			25	3.5		
Penicillium frequentans			50	6.4				
Penicillium funiculosum	25	2.7						
Penicillium luteum					25	3.5		
Penicillium restrictum					25	3.5		
Penicillium rugulosum			25	3.2				
Penicillium viridicatum			25	3.2				
Sterile Mycelia	75	16.2	50	12.9	50	7.1	25	10
Trichoderma viride			25	3.2	25	3.5		
Verticillium candelabrum	25	2.7						
Total Isolates	37		31		28		10	
Total Species	12		14		14		6	

TABLE 69

Site 5 - Assay for May 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia orchidis			25	6.4			50	40
Absidia spinosa			25	3.2				
Alternaria humicola	25	9						
Alternaria tenuis	50	4.5						
Aureobasidium pullulans	50	9	25	6.4				
Cladosporium cladosporioides	50	6.8	25	3.2				
Coniothyrium fuckelii	25	2.2						
Cylindrocarpon candidum	50	4.5	25	3.2				
Cylindrocarpon heteronemum	25	2.2						
Epicoccum purpurascens	25	2.2						
Fusarium culmorum	25	2.2						
Gliocladium roseum	25	2.2			25	11.1	50	20
Mucor microsporus	50	20.4						
Penicillium canescens	25	2.2						
Penicillium decumbens	25	2.2	25	3.2				
Penicillium javanicum	25	2.2						
Penicillium lapidosum	25	2.2	25	6.4				
Penicillium nigricans	25	2.2	25	3.2				
Penicillium ochro-chloron					50	11.1		
Penicillium simplicissimum	25	2.2	50	6.4	25	5.5	25	10
Penicillium wortmanni					25	11.1		
Phoma glomerata	25	2.2	25	3.2			25	10
Sterile Mycelia	25	4.5	50	12.9	75	16.6	25	10
Torula lucifaga	25	2.2						
Trichoderma viride	50	9	100	41.9	100	33.3		
Yeast Species	25	2.2			50	11.1		
Total Isolates	44		31		18		10	
Total Species	22		12		7		5	

TABLE 70

Site 5 - Assay for July 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia spinosa	25	1.7			25	9.6	50	15
Alternaria humicola	100	7.1						
Alternaria tenuis	50	5.3						
Arthrinium phaeospermum	25	1.7						
Aureobasidium pullulans	25	1.7	25	2.3				
Cephalosporium asperum					25	3.2		
Cylindrocarpon candidum	25	1.7			25	3.2		
Cylindrocarpon olidum	25	1.7	25	2.3	50	6.4	25	5
Epicoccum purpurascens	25	1.7						
Eupenicillium species			25	2.3				
Fusarium culmorum					25	3.2	25	10
Fusarium sambucinum var. caeruleum	50	7.1						
Fusarium solani	25	1.7						
Fusarium species	25	1.7			25	6.4	25	5
Gliocladium roseum	50	5.3	75	9.3	25	6.4	25	5
Mucor corticolus	25	3.5	25	2.3			25	5
Mucor hiemalis	50	5.3	75	13.9	25	3.2	25	10
Mucor varians	50	5.3	25	2.3				
Penicillium canescens			25	2.3				
Penicillium cyclopium	50	7.1	50	4.6	25	3.2		
Penicillium decumbens					25	3.2		
Penicillium fellutanum							25	15
Penicillium frequentans					25	3.2		
Penicillium janthinellum					25	3.2		
Penicillium jenseni			25	2.3				
Penicillium lilacinum							25	5
Penicillium meleagrinum					25	6.4		
Penicillium nigricans					25	3.2		

TABLE 70

Site 5

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Penicillium notatum					25	6.4		
Penicillium simplicissimum					25	3.2		
Penicillium urticae	25	1.7						
Penicillium velutinum			25	2.3				
Phoma glomerata	25	3.5						
Phoma herbarum	50	3.5	25	2.3			25	5
Sporotrichum olivaceum	25	1.7						
Sterile Mycelia	25	3.5	50	6.9	50	6.4	50	10
Torula herbarum	25	3.5	25	2.1				
Trichoderma viride	100	17.8	100	27.9	75	12.9	25	10
Verticillium puniceum					25	3.2		
Yeast species	25	1.7			25	3.2		
Zygorhunchus moelleri	25	1.7	25	13.9				
Total Isolates	56		43		31		20	
Total Species	25		16		20		12	

TABLE 71

Site 5 - Assay for September 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	25	7.1			75	20.5		
Absidia spinosa	50	28.5			50	5.8	50	15.7
Alternaria tenuis	25	7.1	25	2.8	25	5.8	25	2.6
Aureobasidium pullulans					25	2.9	50	5.2
Cephalosporium acremonium			25	2.8				
Coniothyrium fuckelii					25	2.9		
Cylindrocarpon olidum					50	5.8	50	7.8
Epicoccum purpurascens					25	2.9		
Fusarium culmorum			50	5.7	50	5.8	25	2.6
Fusarium sambucinum var. caeruleum					25	2.9		
Fusarium solani			100	17.1	25	2.9	25	2.6
Geomyces vulgaris			25	5.7	25	2.9	50	5.2
Gliocladium roseum	25	7.1	25	17.1			50	7.8
Mucor hiemalis	50	28.5			75	14.7	75	7.8
Mucor microsporus					25	2.9		
Penicillium canescens			100	17.1			25	2.6
Penicillium citrinum			25	2.8				
Penicillium fellutanum			50	8.5			50	5.2
Penicillium janthinellum			25	2.8				
Penicillium lilacinum					25	2.9	25	5.2
Penicillium melinii			25	2.8				
Penicillium nigricans					25	2.9	25	5.2
Penicillium purpurogenum							25	2.6
Penicillium restrictum			25	2.8			25	2.6
Penicillium simplicissimum			25	2.8				
Penicillium vermiculatum	25	14.2						
Scopulariopsis brevicaulis							50	5.2

TABLE 71

Site 5

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Spicaria violacea			25	2.8				
Sterile Mycelia					25	2.9	25	2.6
Trichoderma viride	25	7.1	25	8.5	25	8.8	50	7.8
Verticillium candelabrum					25	2.9		
Total Isolates	14		35		34		38	
Total Species	7		15		18		18	

TABLE 72

Site 5 - Assay for November 1971

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	75	33.3	100	29.4	50	13.6	75	18.7
Absidia spinosa			25	2.9	25	9		
Aspergillus fumigatus					25	9		
Aspergillus glaucus			25	2.9				
Botrytis cinerea					25	4.5		
Coniothyrium fuckelii			25	2.9				
Eupenicillium species			25	2.9				
Fusarium culmorum	25	5.5						
Mucor hiemalis	50	11.1	25	2.9	25	18.1	75	56.2
Mucor microsporus	25	11.1	25	2.9	25	4.5		
Penicillium canescens			25	5.8	25	9		
Penicillium chermisinum							25	6.2
Penicillium cyclopium	25	11.1	50	11.7	25	4.5		
Penicillium decumbens	25	11.1	25	11.7	50	9	50	18.7
Penicillium fellutanum			50	5.8				
Penicillium janthinellum	25	5.5	25	5.8				
Penicillium nigricans			50	11.7				
Penicillium purpurescens					25	4.5		
Penicillium urticae	25	5.5						
Penicillium viridicatum	25	5.5						
Phoma herbarum					25	4.5		
Sterile Mycelia					50	9		
Total Isolates	18		34		22		16	
Total Species	9		13		12		4	

TABLE 73

Site 5 - Assay for January 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea	75	17.6	25	16.6				
Alternaria tenuis							25	6.2
Aspergillus amstelodami							25	12.5
Aspergillus glaucus			25	8.3	25	12.5		
Cephalosporium acremonium			25	8.3				
Coniothyrium fuckelii	25	5.8					25	6.2
Gliocladium roseum			25	8.3			25	6.2
Helminthosporium sativum					25	12.5		
Mucor hiemalis							25	6.2
Penicillium brevi-compactum					25	12.5		
Penicillium canescens	25	5.8	25	8.3				
Penicillium claviforme							25	12.5
Penicillium cyclopium	25	5.8	50	16.6	25	12.5	25	6.2
Penicillium decumbens	25	5.8						
Penicillium frequentans					25	12.5		
Penicillium funiculosum	25	5.8						
Penicillium janthinellum	25	5.8	25	8.3				
Penicillium meleagrinum	25	5.8	25	8.3			25	6.2
Penicillium multicolor			25	8.3				
Penicillium ochraceum	25	5.8						
Penicillium restrictum							25	6.2
Penicillium rugulosum							25	6.2
Penicillium spinulosum							25	6.2
Penicillium thomii	25	5.8						
Sterile Mycelia	25	11.7	25	8.3			50	12.5
Trichoderma viride	75	17.6			25	25	25	6.2
Verticillium candelabrum					25	12.5		
Total Isolates	17		12		8		16	
Total Species	12		10		7		13	

TABLE 74

Site 5 - Assay for March 1972

SPECIES	TOP SOIL		5cm		15cm		25cm	
	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.	% Occ.	% Freq.
Absidia coerulea			25	14.2				
Absidia repens	25	2.1						
Absidia spinosa	75	23.9	50	10.7	25	10		
Aspergillus fumigatus	75	2.1	25	7.1	25	10	25	50
Coniothyrium fuckelii			25	3.5	50	10		
Fusarium culmorum	25	2.1	25	3.5				
Fusarium sambucinum var. caeruleum					25	5		
Fusarium solani	75	6.5	25	7.1	25	5		
Gliocladium roseum					25	10		
Mucor hiemalis	50	19.5			25	5		
Mucor racemosus					25	5		
Mucor varians	50	13						
Penicillium citrinum					25	5		
Penicillium cyclopium	25	2.1						
Penicillium expansum			25	3.5				
Penicillium frequentans	25	2.1						
Penicillium funiculosum							25	16.6
Penicillium janthinellum			25	7.1	25	5		
Penicillium luteum	25	2.1						
Penicillium multicolor			50	7.1				
Penicillium nigricans			50	7.1				
Penicillium notatum							25	16.6
Penicillium rugulosum	25	2.1						
Penicillium velutinum	50	8.6	75	17.8	50	10	25	16.6
Periconia macrospinosa	25	2.1						
Sterile Mycelia	25	2.1	25	3.5	25	5		
Trichoderma viride	50	8.6	25	7.1	50	10		
Verticllium psalliotae					25	5		
Total Isolates	46		28		20		6	
Total Species	15		13		14		4	

TABLE 75

Lead analysis pH and soil moisture measurements of soil samples from Site 5

Sample		Lead analysis		Top Soil		5cm		15cm		25cm	
		HAC Pb	HF/HCl Pb	pH	% H_2O	pH	% H_2O	pH	% H_2O	pH	% H_2O
May	1970	400	3,260	7.4	15	7.7	12	7.4	22	7.2	23
July		1,800	3,100	8.5	21	8.5	21	8.5	20	8.5	19
September		250	2,000	7.3	24	7.5	37	7.3	26	7.4	25
November		250	2,000	6.8	35	6.8	35	6.9	36	6.9	29
January	1971	250	2,400	7.1	40	7.0	26	6.9	33	7.0	30
March		350	3,800	7.4	18	7.3	29	7.3	27	7.4	28
May		300	4,400	7.4	17	7.4	26	7.4	26	7.4	24
July		600	2,100	7.4	20	7.4	28	7.0	15	7.3	20
September		900	1,600	7.1	24	7.4	25	7.3	25	7.4	22
November		300	1,700	7.6	34	7.3	31	7.3	24	7.2	29
January	1972	300	1,600	7.8	4	7.5	12	7.5	13.5	7.8	15.5
March		250	1,600	7.9	22	8.1	36.5	7.9	35.5	8.0	28.5
May		250	2,400	7.5	15	7.5	21	7.4	25	7.5	23

Table 76

List of fungi isolated during this investigation

PHYCOMYCETES

Absidia butleri Lendner

Absidia coerulea Bain.

Absidia glauca Hagem

Absidia heterospora Ling Young

Absidia orchidis (Vuill.) Hagem

Absidia repens (Corda) Sacc.

Absidia spinosa Lendner

Mortierella jenkini (Smith) Naumov

Mucor abundans Povah

Mucor corticolus Hagem

Mucor globosus Fischer

Mucor griseo-lilacinus Povah

Mucor hiemalis Wehmer

Mucor jansseni Lendner

Mucor lausanensis Lendner

Mucor microsporus Namyslowski

Mucor racemosus Fres.

Mucor spinescens Lendner

Mucor subtillissimus Oud.

Mucor varians Povah

Mucor species

Rhizopus arrhizus Fischer

Rhizopus oryzae Went & Gerlings

Zygorhynchus heterogamus Vuill.

Zygorhynchus moelleri Vuill.

Zygorhynchus vuilleminii Namyslowski

ASCOMYCETES

Ascomycetous species

Chaetomium funicola Cooke

Chaetomium globosum Kunze

Chaetomium homopilatum Omvik

Chaetomium indicum Corda

Chaetomium olivaceum Cooke & Ellis

Emericellopsis species

Eupenicillium species

Sordaria fimicola (Rob) Ces. & de Not

FUNGI IMPERFECTI

Alternaria fasciculata Cooke & Ellis

Alternaria humicola Oud.

Alternaria tenuis Nees

Arthrinium phaeospermum (Corda) Ellis

Aspergillus amstelodami Thom & Church

Aspergillus aureolus Fennell & Raper

Aspergillus chevalieri (Mang) Thom & Church

Aspergillus chevalieri var. intermedius Thom & Raper

Aspergillus fumigatus Fres.

Aspergillus glaucus Link.

Aspergillus niger Van Tiegh

Aspergillus ochraceus Wilhelm

Aspergillus repens de Bary

Aspergillus sulphureus (Fres.) Thom & Church

Aspergillus sydowii (Bain. & Sart.) Thom & Church

Aspergillus terreus Thom

Aspergillus versicolor (Vuill.) Tirab.

Aspergillus species

Aureobasidium pullulans (de Bary) Arnaud

Botrytis cinerea Pers. ex Fr.

Botryotrichum atrogriseum Van Beyma

Cephalosporium acremonium Corda

Cephalosporium asperum Marchal

Cephalosporium curtipes Sacc.

Cephalosporium humicola Oud.

Cephalosporium roseo-griseum Saksena

Cephalosporium species

Cladosporium cladosporioides (Fres.) de Vries

Cladosporium herbarum (Pers.) Link ex Fr.

Coniothyrium fuckelii Sacc.

Cylindrocarpon candidum (Link) Wollenw.

Cylindrocarpon heteronemum (Berkeley & Broome) Wollenw.

Cylindrocarpon olidum Wollenw.

Doratomyces stemonitis Corda

Eladia saccula (Dale) Smoth

Epicoccum purpurascens Ehrenb. ex Schlecht

Fusarium culmorum (W.G.Sm.) Sacc.

Fusarium oxysporum Schlecht

Fusarium sambucinum var. caeruleum Wollenw.

Fusarium solani (Mart.) Sacc.

Fusarium species

Geomyces vulgaris Traaen

Geotrichum candidum Link

Geotrichum species

Gliocladium penicilloides Corda

Gliocladium roseum Bain.

Gliocladium salmonicolor Raillo

Graphium penicilloides Corda

Helminthosporium sativum Pammel, King & Bakke

Humicola brevis (Gilman & Abbott) Gilman

Mammaria echinobotrys Ces.

Monodictys levis (Wiltshire) Hughes

Monilia geophila Oud.

Nodulisporium species

Oospora sulphurea (Preuss) Saccardo & Voglino

Oospora variabilis (Lindner) Lindau

Oospora species

Paecilomyces carneus (Duche & Heim) Brown & Smith

Paecilomyces striatosporus Onions & Barron

Paecilomyces varioti Bain.

Paecilomyces species

Penicillium aculeatum Raper & Fennell

Penicillium brevi-compactum Dierckx

Penicillium canescens Sopp.

Penicillium chermisinum Biourge

Penicillium chrysogenum Thom

Penicillium citreo-viride Biourge

Penicillium citrinum Thom

Penicillium claviforme Bain.

Penicillium commune Thom

Penicillium corymbiferum Westl.

Penicillium crustosum Thom

Penicillium cyaneo-fulvum Biourge

Penicillium cyclopium Westl.

Penicillium daleae Zal.

Penicillium decumbens Thom

Penicillium diversum Raper & Fennell

Penicillium duclauxi Delacroix

Penicillium expansum Link

Penicillium godlewski Zal.

Penicillium fellutanum Biourge

Penicillium frequentans Westl.

Penicillium funiculosum Thom

Penicillium humuli Van Beyma

Penicillium implicatum Biourge

Penicillium janthinellum Biourge

Penicillium javanicum Van Beyma

Penicillium jenseni Zal.

Penicillium lanosum Westl.

Penicillium lapidosum Raper & Fennell

Penicillium lilacinum Thom

Penicillium luteum Zukal.

Penicillium martensii Biourge

Penicillium megasporium Orpurt & Fennell

Penicillium meleagrinum Biourge

Penicillium melinii Thom

Penicillium multicolor Grigorieva-Manoilova & Poradielova

Penicillium nigricans (Bain.) Thom

Penicillium notatum Westl.

Penicillium ochraceum (Bain.) Thom

Penicillium ochro-chloron Biourge

Penicillium oxalicum Currie & Thom

Penicillium palitans Westl.

Penicillium paxilli Bain.

Penicillium piceum Raper & Fennell

Penicillium piscarium Westl.

Penicillium puberulum Bain.

Penicillium purpurrescens (Sopp.) n. comb

Penicillium purpurogenum Stoll

Penicillium raistrickii Smith

Penicillium ramigena series

Penicillium restrictum Gilman & Abbott

Penicillium roseo-purpureum Dierckx

Penicillium rubrum Stoll

Penicillium rugulosum Thom

Penicillium spinulosum Thom

Penicillium simplicissimum (Oud.) Thom

Penicillium tardum Thom

Penicillium terrestre Jensen

Penicillium thomii Maire

Penicillium urticae Bain.

Penicillium variabile Sopp.

Penicillium velutinum Van Beyma

Penicillium vermiculatum Dangeard

Penicillium viridicatum Westl.

Penicillium waksmani Zal.

Penicillium wortmanni Klocker

Penicillium species

Periconia macrospinosa Lefebvre & A.G. Johnson

Pestalotia pezizoides de Not

Phoma glomerata (Corda) Wollenw. & Hochapfel

Phoma herbarum Westend

Phoma humicola Gilman & Abbott

Phoma violacea (Bertel) Eveleigh

Phoma species

Phomopsis species

Preussia vulgare (Corda) Cain

Pyrenochaeta decipiens Marchal

Scopulariopsis brevicaulis Bain.

Scopulariopsis constantini Bain.

Sepedonium chrysospermum (Bulliard) Fries

Sphaeronaema spinella Kalchbrenner

Spicaria violacea Abbott

Sporotrichum chlorinum Link

Sporotrichum olivaceum Fries

Sporotrichum roseolum Oud. & Beijerinck

Stemphylium botryosum Wallr.

Sterile Mycelia

Torula herbarum (Pers.) Link ex Fr.

Torula lucifaga Oud.

Trichoderma album Preuss

Trichoderma viride Pers. ex fr.

Verticillium bulbillosum Gams & Malla

Verticillium candelabrum Bonordon

Verticillium cinnabarina (Corda) Reinke & Berthold

Verticillium effusum Otth.

Verticillium psalliotae Treschow

Verticillium puniceum Cooke & Ellis

Verticillium sulphurellum Sacc.

Verticillium terrestre (Link) Lindau

YEASTS

Candida albicans (Robin) Berkh.

Candida species

Sporobolomyces roseus Kluyver & Van Niel

Yeast species

Table 77

The effect of several concentrations of lead on growth of Aspergillus fumigatus Fres. (Growth expressed in mm)

Day	Control	Lead Concentration (p.p.m.)								
		6.25	12.5	25	50	75	100	200	300	400
1	10	6	12	7	5	3	5	2	0.5	2
2	24	12	22	15	12	13	13	10	5	4
3	35	21	31	25	20	20	18	16	8	11
4	47	31	42	35	30	29	27	22	12	18
5	55	41	53	42	40	35	32	26	19	25
6	66	53	58	51	45	41	35	31	24	28

Table 78

The effect of a range of lead concentrations on colony diameter of Aspergillus fumigatus Fres.
(Growth expressed in mm)

	Concentration of Lead (in p.p.m.)																
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	Control
DAY 1	6	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	10
2	10	7	4	3	3	3	3	3	3	3	3	3	3	3	3	3	23
3	17	12	8	7	4	4	4	4	4	3	3	3	3	3	3	3	34
4	32	23	14	12	7	6	6	5	4	4	4	4	4	4	3	3	44
5	44	30	17	13	7	10	8	7	7	5	5	5	4	4	3	3	50
6	59	36	18	16	8	12	11	9	11	6	5	6	4	5	4	4	62
7	64	39	19	18	11	12	14	15	13	10	7	8	5	6	4	4	71
8	79	45	21	21	14	13	18	20	17	12	8	11	5	7	4	5	82

Table 79

Average weight of Aspergillus fumigatus in Czapek Dox medium of different pH

pH	Weight (mg)
2	15
2.5	94
3	214
3.5	214
4	178
4.5	245
5	169
5.5	250
6	262
6.5	276
7	227
7.5	194
8	202

Table 80

Growth of A. fumigatus in a range of lead concentrations

Concentration	Weight in milligrams	
	Day 4	Day 16
Control	41	188
50	28	209
100	30	168
150	26	23
200	13	29
250	27	31
300	17	26
350	10	32
400	11	33
500	30	38
600	25	36

Table 81

Growth of Aspergillus fumigatus in a range of lead concentrations

Lead Concentrations (p.p.m.)														
Days	Control	100	110	120	130	140	150	160	170	180	190	200	210	220
0	19	22	19	16	18	19	20	21	22	22	19	16	17	17
2	20	23	22	20	20	20	20	21	20	24	20	20	24	17
4	33	26	29	27	25	26	27	27	28	30	30	36	32	31
6	61	17	23	23	21	24	22	21	22	22	22	20	22	20
8	153	30	23	30	23	23	23	24	24	30	29	18	17	24
10	166	48	46	42	31	34	28	26	29	26	25	19	20	22
12	174	56	38	56	35	32	27	29	28	22	25	24	27	25
14	168	87	85	85	62	57	42	29	28	25	26	27	28	25
16	190	94	85	80	64	51	43	42	39	33	30	34	36	36
18	188	167	132	158	105	84	43	52	61	30	21	25	27	23
20	208	147	148	142	103	80	55	60	35	41	35	32	30	36
22	213	137	139	127	91	77	85	63	34	40	28	22	30	22
24	213	147	121	134	96	103	107	93	30	27	37	37	27	28
26	228	208	200	167	132	106	87	102	47	43	30	33	28	30
28	232	220	184	185	139	114	84	97	45	44	31	46	24	29
30	219	192	203	194	118	130	93	109	68	43	37	32	30	31

Table 82

Growth of A. fumigatus in solutions without lead and solutions with lead and transferred from lead to lead free medium

Weight (in mg)

Day	Lead free		Lead containing		
	At transfer	After transfer	At transfer	After transfer to lead containing	After transfer to lead-free
4	34	252	22	140	260
6	61	258	28	180	240
8	119	240	22	160	210
10	130	270	27	114	256

Table 83

Growth of Aspergillus fumigatus in media containing various amino acids with and without lead

Amino Acid	Weight in mg		t test
	Control	160 p.p.m. Pb	
Czapek Dox only (1)	228	118	xxx
Threonine (3)	236	142	xx
Methionine	211	156	x
Serine	242	157	xx
Cysteine (5)	280	106	xx
Tryptophan	216	166	xxx
Leucine	245	247	N.S.
Norvaline	196	207	N.S.
Alanine	194	238	N.S.
Isoleucine	227	272	N.S.
Valine	177	238	N.S.
Phenylalanine	206	254	N.S.
Citrulline	170	211	N.S.
Dihydroxyphenylalanine	221	323	N.S.
Ornithine	238	227	N.S.
Proline	248	226	N.S.
Aspartic acid	237	211	N.S.
Histidine	309	196	N.S.
Glycine	266	183	N.S.
Lysine	297	297	N.S.
Glutamic acid	273	268	N.S.
Cystine	247	260	N.S.
αAminobutyric acid (2)	204	323	xxx
Norleucine (4)	252	375	xx
Tyrosine	273	316	xx
Arginine	245	338	xx

x = p = 0.05

xx = p = 0.01

xxx = p = 0.001

Table 84

Growth of various isolates of A. fumigatus in the presence of lead after 20 days

Isolate	Control	110 p.p.m.	160 p.p.m.
Spoil	241	319	101
96202	246	300	189
16062	260	282	188
94164	244	210	185
35570	294	225	121
28646	299	310	119
89354	239	252	114
45338	258	308	111
69714	244	238	228
89353	162	208	221
108008	231	244	269
121660	146	172	255

Table 85

Growth of various species and their isolates in 160 p.p.m. lead

Species	Control	160 p.p.m.		t test
		Spoil	C.M.I.	
Gliocladium roseum	328	182	248	xx
Verticillium psalliotae	321	214	186	NS
Cylindrocarpon olidum	307	197	127	x
Phoma herbarum	294	132	35	xxx
Absidia coerulea	258	171	35	xxx
Penicillium frequentans	258	164	250	xxx
Fusarium sambucinum var. caeruleum	250	157	240	x
Penicillium simplicissimum	240	18	34	xxx
Cephalosporium acremonium	201	102	113	NS
Trichoderma viride	196	190	202	NS

x = p = 0.05

xx = p = 0.01

xxx = p = 0.001

Table 86

Growth of several species in 200 p.p.m. lead and Control Solutions

Species	Weight (mg)	
	200 p.p.m. Pb	Control
Aspergillus fumigatus Spoil	32	241
A. fumigatus 69714	15	244
A. fumigatus 89353	18	162
A. fumigatus 108008	16	231
A. fumigatus 121660	15	146
Verticillium psalliotae Spoil	151	321
Cylindrocarpon olidum Spoil	115	307
Absidia coerulea Spoil	17	258
Fusarium sambucinum var. caeruleum Spoil	32	250
Cephalosporium acremonium Spoil	42	201

Table 87

Average weight in mg of 3 flasks of the fungi at transfer

Fungus	transfer								
	1	2	3	4	5	6	7	8	9
Aspergillus fumigatus	264	210	217	266	262	260	213	283	282
A. fumigatus 108008	206	267	258	286	212	256	248	246	226
A. fumigatus 121660	46	145	168	198	146	199	212	196	182
Verticillium psalliotae	159	322	328	338	307	321	332	283	270
Fusarium sambucinum var. caeruleum	95	204	146	193	216	170	130	146	134

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